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COCCIDIOSIS OF THE LIVER IN RABBITS

I. EXPERIMENTAL STUDY ON THE EXCYSTATION OF OOCYSTS OF EIMERIA STIEDAE

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NEW YORK

The mode of liberation of sporozoites from oocysts of Eimeria stiedae has been studied by many workers; certain discrepancies in the results, however, made it desirable to take up this problem again and to determine the conditions and factors necessary for bringing about excystation.

THE LITERATURE

The early investigators in this field (Rieck,[1] R. Pfeiffer [2]) conducted experiments with the natural gastric and intestinal juices of animals, to which they added oocysts, studying the changes in the membranes which took place in these mediums. While these authors limited their observations chiefly to the changes in the membranes of oocysts, Metzner [3] studied the influence of gastric and intestinal juices on the sporozoites themselves. After repeating the experiments of Rieck and Pfeiffer with gastric juice, he came to the conclusion that the exposure of oocysts to gastric juice did not cause the liberation of sporozoites, although swelling, folding and rupture of the membranes of some of the cysts could be observed. He then placed oocysts in the duodenal juice of rabbits at 37 C., and was able to watch the process of liberation of sporozoites under the microscope after two hours' exposure. Not satisfied with the fact that duodenal juice causes the liberation of sporozoites from the oocysts, Metzner then tried to obtain intestinal content composed chiefly of pancreatic juice by collecting the juice from the duodenum at the point of entrance of the pancreatic duct after the injection of secretin. Since the effect of this "secretin-duodenal juice" was much better than that of plain duodenal juice, he concluded that pancreatic juice alone may be instrumental in bringing about the liberation of sporozoites. In order to furnish further proof of this point he then used aqueous solutions of chemical preparations of pancreas of different animals, which were also effective in causing excystation of sporozoites. With regard to the observations of later workers in this field it should be stated that Metzner did not consider the swelling and folding of the membranes of oocysts which occur in certain mediums as criteria of the liberating action of the medium on the oocysts; on the contrary, he stated that these changes are usually observed in mediums not suited to excystation.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, and the Department of Chemistry of the New York State Psychiatric Institute and Hospital.

1. Rieck: Deutsche Ztschr. f. Tiermed. u. vergl. Path. **14**:52, 1889.

2. Pfeiffer: Die Coccidienkrankheit der Kaninchen, Beitr. zur Protozoenforschung, Berlin, A. Hirschwald, 1892.

3. Metzner: Arch. f. Protistenk. **2**:51, 1903.

Metzner's observations on the liberating effect of duodenal juice and chemical preparations of pancreas were confirmed by Reich,[4] Lerche,[5] Waworuntu[6] and Dieben,[7] although most of these authors disagreed as to the time of exposure of the oocysts to duodenal juice or pancreatic preparations necessary to effect the liberation of sporozoites. According to their statements, excystation takes place after from seven to seventeen hours' exposure of oocysts to duodenal juice or pancreatic preparations.

In discussing this question, Krijgsman[8] pointed out that while Metzner worked with oocysts of Eimeria stiedae obtained from hepatic lesions and from the gall-bladder, the later authors used oocysts of Eimeria stiedae collected from the feces, and he ascribed the difference in the results to the different types of oocysts. Krijgsman, using, as criterion of excystation, the folding, swelling and rents of oocyst membrane, also found a greater and more rapid effect after successive exposure of oocysts to gastric and duodenal juice than after exposure to duodenal juice or pancreatic preparations alone.

Excystation in vivo was carried out by Andrews,[9] who fed oocysts to young rats by stomach tube and examined the intestinal contents of these animals one or more hours after feeding.

In repeating his experiments with rabbits from 2 to 3 weeks old, I found this an excellent method. For my purposes, however, the study of excystation in vitro seemed more desirable.

MATERIAL, METHODS AND TECHNIC

Oocysts.—Oocysts of Eimeria stiedae,[10] collected from the feces as well as from lesions of the liver, were used exclusively in these studies. Oocysts from the feces were obtained by the following method: The droppings of experimentally infected rabbits were collected in 1 per cent chromic acid solution, ground in a mortar, and then put in shallow dishes in order to allow sporulation of the oocysts to take place. The oxygen necessary for sporulation can also be supplied by bubbling air through the flask containing oocysts and 1 per cent chromic acid solution; by this method large quantities of material can be handled without taking precautions concerning evaporation of the fluid.

After completion of the sporulation, the oocysts were freed from the débris by the flotation method,[9] saturated saline solution being used. The oocysts were then washed with sterile distilled water, placed in sterile saline solution and kept in the icebox. Oocysts obtained from the feces will be termed L C F in the tables.

Oocysts from lesions in the liver were obtained by cutting the organ into small pieces, which were then passed through a meat-grinder. The ground material was then screened and collected in 1 per cent chromic acid solution, and after the com-

4. Reich: Arch. f. Protistenk. **28**:1, 1912.

5. Lerche: Arch. f. Protistenk. **42**:380, 1921.

6. Waworuntu: Bijdrage tot de kennis van het kanignencoccidium, Dissertation, Utrecht, 1924.

7. Dieben: Over de morphologie en biologie von het rattencoccidium Eimeria Nieschulzi en zyn verspreiding in Nederland, Dissertation, Utrecht, 1924.

8. Krijgsman: Arch. f. Protistenk. **56**:116, 1926.

9. Hegner and Andrews: Problems and Methods of Research in Protozoology, New York, The Macmillan Company, 1930.

10. The material for the initial infection was given to me by Dr. W. G. Bachman, professor of parasitology at the School of Tropical Medicine in San Juan, Porto Rico.

pletion of sporulation the oocysts were separated from the débris by the flotation method. Such oocysts will be termed L C in the tables.

Mediums.—1. Gastric Juice: One hour after giving about 50 cc. of saline solution by stomach tube to a rabbit of medium size, the animal was put to death. The stomach was then removed, its contents centrifugated and the supernatant fluid filtered through a Berkefeld filter N.

2. Intestinal Juice: The small intestine of a rabbit was ligated at both ends and about 50 cc. of saline solution injected into the lumen. The contents of the intestine were then transferred to a vessel and centrifugated, and the supernatant fluid was filtered through a Berkefeld filter N.

3. Pancreatic Juice: A pancreatic fistula was produced by inserting a cannula into the distal portion of the pancreatic duct, and the sterile pancreatic juice was collected in a rubber bag attached to the tube draining the duct. Large rabbits weighing from 7 to 8 pounds (3.2 to 3.6 Kg.) were used for this operation.

Sterile pancreatic juice of dogs was given to me repeatedly by Dr. B. N. Berg, to whom I am greatly indebted for this contribution.

The following commercial preparations of enzymes were used: pepsin, U.S.P., Eimer & Amend; trypsin, Fairchild Bros. & Foster; trypsin, Eimer & Amend; pancreatin, U.S.P., Armour & Co.; pancreatin, U.S.P., Eimer & Amend; steapsin, Eimer & Amend; takadiastase, Parke, Davis and Co.; preparations of papain and enterokinase were given to me by Dr. E. Brand.[11]

For all experiments on excystation of oocysts with solutions of chemical preparations of pancreatic enzymes, 5 per cent solutions were used; that is, 5 Gm. of the powder was put into 100 cc. of distilled water and stirred until the material was well suspended. After filtering through a paper filter, the solution was filtered through a Berkefeld filter N, in order to avoid bacterial growth, and was kept in the icebox. The use of chemicals, for instance, toluene, added to solutions of enzymes in order to prevent bacterial growth, is not permissible, since its presence leads to degenerative changes in sporozoites within oocysts.

Likewise, glycerin produces a damaging effect on oocysts; therefore, glycerin extracts of pancreas or of intestinal mucosa could not be used for the studies on excystation.

The activity of each enzyme preparation was determined according to the methods described by Willstaetter, Waldschmidt-Leitz and their co-workers.[12] The enzymatic activity is expressed in the tables as the number of cubic centimeters of fifth-normal alcoholic potassium hydroxide necessary to neutralize the increase in acidity due to the action of 1 cc. of the enzyme solution on casein, on leucylglycine or on olive oil after subtraction of the blank. The effect of amylase on starch is calculated in milligrams of maltose formed by 1 cc. of solution of enzyme.

Temperature.—All analyses were carried out in incubators at 37 C.

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11. This enterokinase was prepared for Dr. Brand by courtesy of Dr. F. Fenger, director of research of Armour & Co. The intestinal mucosa was dried according to the method of Waldschmidt-Leitz (Ztschr. f. physiol. Chem. **132**:204, 1924); from this powder a purified preparation was obtained according to the method of Waldschmidt-Leitz (ibid. **142**:223 [exper. 4] 1925).

12. Willstaetter, Waldschmidt-Leitz, Dunaiturria and Kuenstner: Ztschr. f. physiol. Chem. **160-161**:196, 1926. Waldschmidt-Leitz and Hartenek, Anna: ibid. **146-147**:286, 1925. Willstaetter and Waldschmidt-Leitz: ibid. **125**:93, 1923. Willstaetter, Waldschmidt-Leitz and Hesse: ibid. **126**:143, 1923.

Hydrogen Ion Concentration of the Medium.—The hydrogen ion concentrations of the various mediums were determined by a method which was satisfactory for readings of an accuracy within from 0.1 to 0.2.

For the purpose of studying excystation, the desired hydrogen ion concentration of each preparation was attained by adding drops of either 1 per cent hydrochloric acid or 2 per cent solution of sodium bicarbonate to the medium. Because of the unfavorable effect of fifth-molar phosphate solutions, no other buffers were used in experiments concerning excystation. For the chemical analyses of the enzymatic action of the mediums, however, the original buffers designed for these experiments were employed.

Methods Used for Studying the Effects of Mediums on Excystation in Vitro.—The mediums were put into sterile test tubes, the hydrogen ion concentrations were adjusted to the desired point, and 1 or more drops of oocysts suspended in saline solution were added. The test tubes were then placed in the incubator at 37 C., and samples of oocysts were aspirated from the sediment after various intervals of exposure. One or two drops of the aspirated material were placed on a slide and covered with a coverslip. The preparation was then studied under the high power lens of the microscope, and 100 sporulated cysts were counted. The number of those showing changes, such as moving sporozoites, free sporozoites within the oocysts and partially or completely empty shells, was registered. The average of three counts was taken as the percentage of effect in a single experiment.

For most of the experiments, 2 cc. of enzyme solution was used, to which 2 drops of a suspension of oocysts in an equal volume of saline solution were added. For each additional drop of oocyst material, 1 cc. of fluid medium was thought to be adequate.

The method of determining the percentage of effect on oocysts naturally could not be very precise. Moreover, the worker had to be familiar with abnormal forms of sporulation and degenerative changes of oocysts which should be excluded from the counts. However, the average of several readings of one experiment taken by an experienced worker can thus be expressed by figures and can be compared with the results of other similar experiments.

THE ENZYME CONCERNED IN EXCYSTATION OF OOCYSTS [13]

Metzner's [3] observations and experiments leave no doubt as to the liberating influence of pancreatic juice on oocysts of Eimeria stiedae. The question which of the pancreatic enzymes is specifically concerned in this process has not, however, been definitely answered.

It is generally assumed that trypsin causes excystation, because solutions of commercial preparations of trypsin are effective. Analyses of the enzymatic properties of commercial preparations of trypsin, however, reveal the presence of various amounts of the other enzymes of the pancreas, which makes it questionable whether trypsin alone is responsible for the liberating activity of these mediums.

The results of such analyses of several commercial preparations of pancreas as well as of pancreatic and duodenal juices and the effects of these mediums on excystation are presented in table 1.

13. The chemical part of this study was carried out in the chemical laboratory of the New York State Psychiatric Institute and Hospital.

From this table it can be seen that trypsin and diastase are present in all the preparations examined; lipase is absent only in taka-diastase. Erepsin can be excluded in all probability as a causative factor of excystation, since preparations showing no demonstrable action of this enzyme effect liberation of sporozoites.

The question whether any enzyme at all is necessary for excystation was answered in the following ways:

1. Oocysts were put into test tubes containing saline solution adjusted with 1 per cent hydrochloric acid and 2 per cent solution of sodium bicarbonate to p_H 6, 6.5, 7, 7.5 and 8, and these test tubes were kept in the incubator at 37 C. for a period of forty-eight hours. During this time of exposure, repeated examinations of these oocysts showed no evidence of excystation.

TABLE 1.—*Comparative Analyses of Pancreatic Preparations and Pancreatic Juices*

Medium	Trypsin: Activated with Enterokinase, Action per Cc. in 20 Min.*	Trypsin: Not Activated, Action per Cc. in 20 Min.*	Erepsin, Action per Cc. in 1 Hr.*	Lipase, Action per Cc. in 1 Hr.*	Diastase, Mg. of Maltose Formed per Cc. in 10 Min.	Excystation, per Cent LC;† p_H 7.5; 18 Hr. Exposure
Trypsin, Fairchild Bros. & Foster	0.98	0.98	0.00	0.10	79.0	32
Pancreatin, Eimer & Amend	0.40	0.44	0.02	1.90	100.3	17
Pancreatin, Armour & Co.	0.50	0.50	0.00	0.23	139.5	23
Steapsin, Eimer & Amend	0.80	0.82	0.00	0.59	150.1	40
Taka-diastase, Parke, Davis & Co.	0.20	0.20	0.00	0.00	116.9	4
Trypsin, Eimer & Amend	0.48	0.32	0.04	2.34	104.9	22
Pancreatic juice, dog	0.65	0.13	0.07	3.20	133.2	40¶
Pancreatic juice, rabbit	0.50	0.15	0.00	3.10	65.2	44¶
Intestinal juice, rabbit	0.39	0.22	0.54	2.00	52.4	52¶

* Expressed as the number of cubic centimeters of fifth-normal alcoholic potassium hydroxide necessary to neutralize the increase in acidity due to the action of 1 cc. of the enzyme solution on casein (trypsin), leucylglycine (erepsin) or olive oil (lipase) after subtraction of the blank.

† Oocysts of Eimeria stiedae collected from lesions of the liver.

¶ Effect of juice activated with enterokinase.

The same negative result was obtained with fifth-molar phosphate buffers at p_H 6 to 8.

2. Pancreatic juices of dogs and rabbits, as well as solutions of commercial preparations of pancreatic enzymes, were heated to 50, 60, 70 and 80 C., in order to damage partly or destroy the enzyme. The effect of these preparations on oocysts was then tested, and in three cases a chemical determination of their tryptic activity was performed. These experiments were carried out as follows:

The different mediums were divided into several portions, which were then separately exposed to heat for one hour in different water baths at 50, 60, 70 and 80 C. One portion that was not heated was used as a control.

Before being heated, the various fluids were adjusted to p_H 6, 7 or 8. After heating, they were readjusted to p_H 7 or 7.5. To each of the test tubes containing 3 cc. of medium, 3 drops of a suspension of oocysts in saline solution were added, and the samples were kept in the incubator at 37 C. for eighteen hours.

The results of these experiments are seen in table 2. Corresponding chemical analyses of the tryptic action on casein of mediums 4, 5 and 6, after their exposure to heat are given in table 3.

From the results of these experiments one may conclude that the active principle causing the liberation of sporozoites is severely damaged by exposure to heat at 60 C. for one hour and is almost completely destroyed at 70 C. The higher the p_H of the medium when it is exposed to heat, the more easily the active principle seems to be destroyed.

3. In another series of experiments, fresh pancreatic juice of a dog was filtered through a collodion membrane [14] under a pressure of about

TABLE 2.—*Influence of Heat on Liberating Action of Medium*

Medium	p_H Before Heating	p_H of Experiment	Type of Oocysts	Excystation, per Cent in 18 Hr. Control Medium, Not Heated	Medium Heated for 1 Hr. to 50 C.	60 C.	70 C.	80 C.
Pancreatic juice, dog	7.0	7.0	LC*	38	31	17	3	2
Pancreatic juice, rabbit	7.0	7.0	LC*	36	37	11	5	1
Intestinal juice, rabbit	7.0	7.0	LC*	40	34	21	17	2
Steapsin, Eimer & Amend	6.0	7.5	LCF†	49	45	38	12	0
Trypsin, Fairchild Bros. & Foster	8.0	7.5	LCF†	32	34	7	0	0
Steapsin, Eimer & Amend	8.0	7.5	LCF†	50	40	30	0	0

* Oocysts of Eimeria stiedae collected from lesions of the liver.
† Oocysts of Eimeria stiedae collected from the feces.

TABLE 3.—*Influence of Heat on Tryptic Action of Medium*

Medium	p_H Before Heating	p_H of Experiment	Tryptic Action per Cc. in 1 Hr.* Control Medium, Not Heated	Medium Heated for 1 Hr. to 50 C.	60 C.	70 C.	80 C.
Steapsin, Eimer & Amend	6.0	8.8	1.00	0.8	0.66	0.10	0.0
Trypsin, Fairchild Bros. & Foster	8.0	8.8	0.96	0.8	0.29	0.12	0.08
Steapsin, Eimer & Amend	8.0	8.8	1.04	0.96	0.48	0.04	0.00

* Expressed as the number of cubic centimeters of fifth-normal alcoholic potassium hydroxide necessary to neutralize the increase in acidity due to the action of 1 cc. of the enzyme solution on casein after subtraction of the blank.

100 mm. of mercury. After the addition of 0.2 cc. of a 0.5 per cent solution of enterokinase to each 1.8 cc. of this ultrafiltrate, the mixture was adjusted to p_H 7.5 and its action on oocysts compared with the effect of the same, activated, nonfiltered juice at p_H 7.5 In addition, analyses of the enzymatic properties of both mediums were performed.

The biuret-free ultrafiltrate never caused excystation, while the nonfiltered pancreatic juice was effective. Only traces of enzymes could be demonstrated in the ultrafiltrate by chemical analyses.

14. The membrane was made of a concentrated preparation of pyroxylin (Schering) 7 Gm., ether 90 cc., absolute alcohol 10 cc. and ethylene glycol 5 cc.; it was approximately 0.2 mm. thick. For details, see Eggerth (J. Biol. Chem. **48**:203, 1921).

From the outcome of these experiments it can be concluded that excystation of oocysts is dependent on the enzymic action of the mediums.

INFLUENCE OF THE HYDROGEN ION CONCENTRATION OF THE MEDIUM ON EXCYSTATION

The next step was to determine the range of hydrogen ion concentration that would permit excystation.

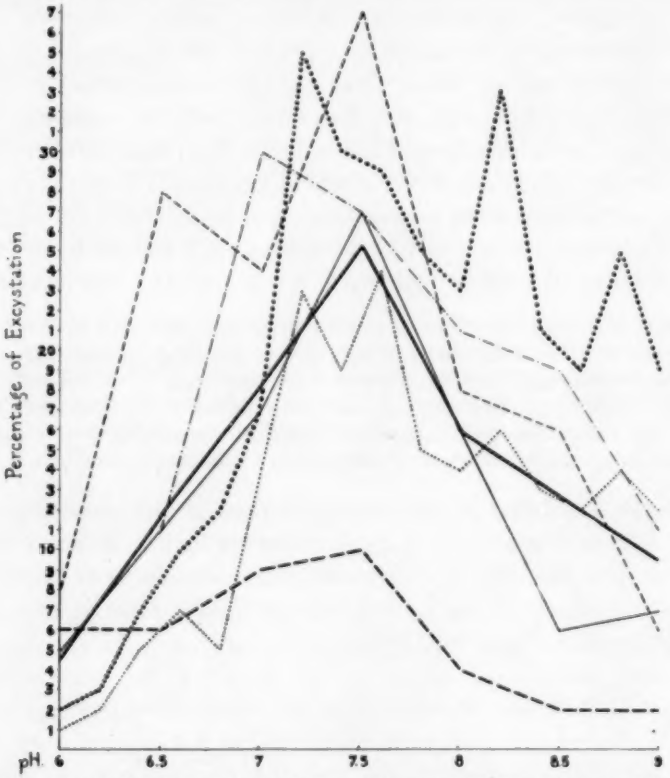

Fig. 1.—The influence of the hydrogen ion concentration of the medium on the excystation of oocysts of Eimeria stiedae collected from the feces of rabbits. The curves represent the percentages of excystation in various mediums with the given hydrogen ion concentrations, as follows: preparations of steapsin (fine dotted line, heavy dotted line, dash and dot line); preparations of trypsin (fine broken line, heavy broken line (purified trypsin) and fine unbroken line. The thick unbroken line represents the averages of all counts in all mediums at the given hydrogen ion concentrations. In figures 1, 2 and 3, each percentage represents an average based on three counts.

For this purpose, solutions of trypsin and steapsin were adjusted to from p_H 6 to p_H 9 by the addition of various numbers of drops of 2 per cent solution of sodium bicarbonate. The difference of p_H between the solutions was arbitrarily

chosen and ranged from 0.2 in some experiments to 0.5 in others. To 2 cc. of medium 2 drops of a suspension of fecal oocysts in saline solution were added, and the test tubes were then placed in the incubator at 37 C. All readings were taken seven hours after exposure.

The results are given in figure 1. The horizontal line represents the hydrogen ion concentrations of the mediums—from p_H 6 to p_H 9; the vertical lines mark the percentages (each an average of three counts) of oocysts showing evidence of excystation. The thick unbroken line represents the averages of all counts taken at p_H 6, 6.5, 7, 7.5, 8, 8.5 and 9.

Although there is considerable variation of individual counts at the same p_H, the average taken from six experiments shows a straight incline from p_H 6 up to p_H 7.5, the curve declining somewhat more slowly toward p_H 9. The actual upper limit of p_H was not determined. Excystation was practically never observed below p_H 5.5, although movements of sporozoites were occasionally seen in solutions of p_H 5.2 to p_H 5.5. Optimum action was found between p_H 7 and p_H 8, and therefore p_H 7.5 was adopted as a standard in practically all the experiments.

Owing to the use of solutions of low buffering capacity, such as hydrochloric acid and sodium bicarbonate, the readings of the hydrogen ion concentrations of the mediums at the end of the experiments were often from 0.2 to 0.8 higher than those at the beginning. In numerous attempts to stabilize the hydrogen ion concentration by using fifth-molar phosphate buffers, the percentages of excystation were so low that the use of these highly buffered solutions had to be abandoned.

Although the hydrogen ion concentration of the pancreatic juice obtained by pancreatic fistula is p_H 8 or slightly higher, it seems reasonable to assume that the liberation of sporozoites in vivo takes place in the neighborhood of p_H 7, since the pancreatic juice is neutralized by the acid-containing gastric juice.

INFLUENCE OF TEMPERATURE ON EXCYSTATION

Only a few experiments were performed to test the influence of the temperature of the medium on the liberation of sporozoites of Eimeria stiedae. Excystation was never observed in suitable mediums kept at room temperature ranging approximately from 24 to 28 C., even after prolonged exposure, while corresponding preparations kept in the incubator at 37 C. showed positive results.

No attempt was made to determine the lower and upper limits of temperature that would permit excystation.

When, however, the excystation of oocysts had commenced at an incubator temperature of 37 C., it could be continued at a much lower temperature. It was often observed that sporozoites of oocysts started to move after the test tubes had been taken out of the incubator and had been kept at room temperature for a long time. After the exposure of the mediums to 37 C. for some time, all stages of

excystation could be followed under the microscope without placing the preparations on a warm stage. In several instances, excystation was even observed after the test tubes had been kept in the icebox for about one hour following their exposure in the incubator to a temperature of 37 C. for several hours.

THE INFLUENCE OF TIME OF EXPOSURE ON EXCYSTATION

Metzner [3] observed beginning excystation as early as one hour after exposure of oocysts to intestinal juice and completion of excystation of many oocysts two hours after their exposure to the same medium at 37 C. Later investigators—Lerche,[5] Waworuntu,[6] Dieben [7] and Krijgsman,[8] on the other hand, maintained that a much longer time of exposure to duodenal juice or solutions of commercial pancreatic preparations is needed in order to effect excystation. In discussing the possible causes of the discrepancies, Krijgsman pointed out two factors that might explain the difference in time required for excystation of oocysts by different authors:

1. The different types of oocysts used for the experiments by different workers:

Metzner used oocysts of Eimeria stiedae obtained from the gallbladder or from lesions in the liver, while the later workers conducted experiments with oocysts of Eimeria stiedae collected from the feces of rabbits. According to Krijgsman, the oocysts obtained from lesions in the liver are immature, and their membranes are therefore less resistant to intestinal digestion than those collected from the feces, which have passed naturally through the bile duct and the intestines.

2. The influence of gastric digestion on oocysts prior to pancreatic digestion.

I tested these statements by three types of experiments as follows:

1. A study of the influence of the time of exposure on excystation of oocysts of Eimeria stiedae obtained from lesions of the liver as well as of oocysts collected from feces of the same animal, in identical mediums with the same hydrogen ion concentration.

Aqueous solutions of trypsin and steapsin of p_H 7.5 were used. To each 5 cc. of medium 8 drops of suspension of oocysts (obtained from lesions of the liver and from feces) in saline solution were added and the test tubes then exposed in the incubator to a temperature of 37 C. Counts of samples were made every hour up to seven hours and finally fourteen hours after exposure.

The results of these experiments are given in figure 2. On the horizontal line are the hours of exposure up to fourteen hours, while the vertical lines mark the percentages (each an average of three counts) of excystation after from one to seven hours and after fourteen hours of exposure.

From figure 2 it can be seen that evidence of excystation was observed in some of the mediums one hour after exposure; after two

hours' exposure, all mediums showed various stages of excystation; up to five hours the percentage of excystation increased steadily, while from seven to fourteen hours only a slight increase of effect was noted. The averages of the individual counts at different hours, represented by the solid thick line, give a fairly good idea of the influence of time on excystation. It shows how important it is to take readings of similar

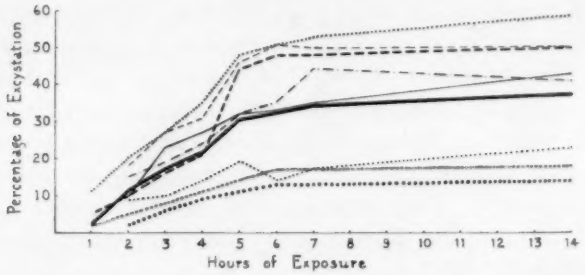

Fig. 2.—The influence of the time of exposure to the medium on the excystation of oocysts of Eimeria stiedae. The curves represent the percentages of excystation after the given periods of exposure to the various mediums, as follows: preparations of trypsin (fine unbroken line and fine dotted line, oocysts from feces being used in the first case and those from hepatic lesions in the second); preparations of steapsin (fine broken line, heavy broken line, and line of crosses, oocysts from feces being used in each case, and dash and dot line, heavy dotted line and line of circles, oocysts from hepatic lesions being used). The thick unbroken line represents the averages of all counts in all mediums at the given times of exposure.

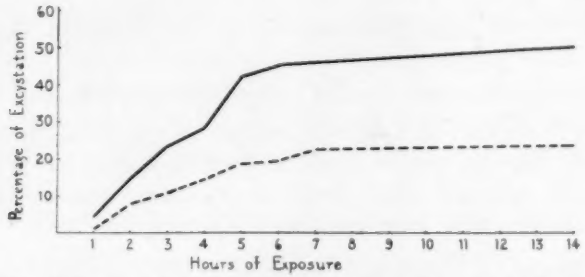

Fig. 3.—The influence of the type of oocyst on the excystation. The curves represent percentages of excystation as follows: the unbroken line, that of oocysts obtained from feces; the broken line, that of oocysts from lesions of the liver.

experiments after the same lengths of time of exposure in order to avoid errors in comparative analyses.

2. A study of the behavior of oocysts of Eimeria stiedae obtained from lesions of the liver and of oocysts collected from the feces of the same animal in identical mediums with the same hydrogen ion concentration.

The figures obtained in the previous experiment were evaluated in a different way, whereby the different behavior of the different types

of oocysts becomes apparent. By plotting the average counts obtained with oocysts from lesions of the liver and with those from the feces separately, two curves are seen, representing the average percentages of excystation of the two types of oocysts in identical mediums after the same length of time of exposure (fig. 3).

It will be noted that, contrary to the assumption of Krijgsman, the oocysts collected from the feces responded more quickly than those obtained from lesions in the liver; likewise, the percentage of excystation of oocysts from the feces was generally higher.

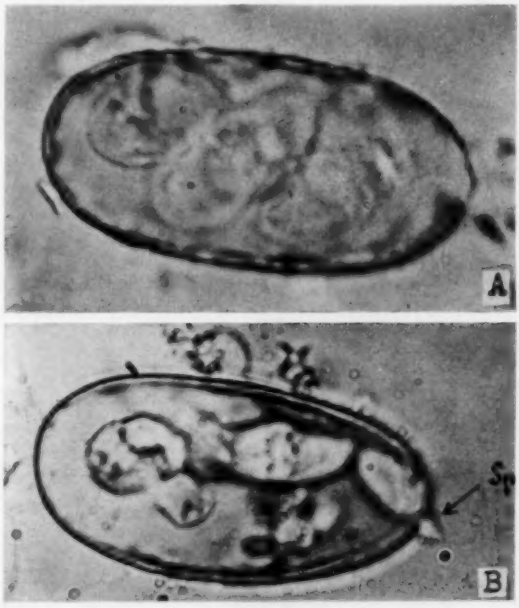

Fig. 4.—*A*, oocyst of Eimeria stiedae after liberation of sporozoites. Note the intact wall of the oocyst enclosing the four intact but empty sporocysts. *B*, oocyst during the process of excystation of sporozoites. *Sp* indicates a sporozoite creeping through the micropyle of the oocyst.

3. A study of the influence of gastric digestion of oocysts from lesions of the liver and of oocysts from feces, prior to their exposure to pancreatic digestion.

It has been pointed out in foregoing paragraphs that Krijgsman [8] considered the influence of gastric digestion of oocysts prior to their intestinal digestion as an important factor with regard to the length of time necessary in bringing about excystation. Unfortunately, from the descriptions of his results [15] one cannot form any clear idea as to the effect of the mediums on excystation, because of his different point of view concerning the criteria of excystation. He described changes

15. Krijgsman: Arch. f. Protistenk. **56**:122 (tables 3 and 4) 1926.

of the membranes, such as folds, swelling and rents, and empty shells, which in my experience are signs of degeneration of oocysts in mediums not suited to excystation. Contrary to Krijgsman's descriptions, I observed, in many of my experiments, partly or completely empty oocysts, showing intact membranes without folds and rents, enclosing faintly visible sporocysts and rest bodies (fig. 4 *A*).

Basing my observations on active movements of sporozoites as a criterion of excystation, I conducted a series of experiments in order to test the influence of gastric digestion prior to pancreatic digestion.

Oocysts of Eimeria stiedae obtained from lesions of the liver, as well as from the feces of the same animal, were exposed to 1 per cent solution of pepsin, U.S.P., in 0.5 per cent hydrochloric acid or to natural gastric juice of rabbits for one hour at 37 C. Afterward the test tubes containing these mediums and oocysts were gently centrifugated for about five minutes, and the sediment was washed with saline solution twice; after removal of the supernatant fluid, 2 cc. of pancreatic juice of the dog (of p_H 7.5) or 2 cc. of an aqueous solution of steapsin of p_H 7.5 was added to the oocysts, and the test tubes were then placed in the incubator. At the same time, oocysts of both types, not previously treated with pepsin or natural gastric juice, were put in the pancreatic juice of p_H 7.5 or into the solution of steapsin of p_H 7.5 and these controls likewise placed in the incubator at 37 C. Readings of all samples were made one hour, two hours and eight hours after exposure.

TABLE 4.—*Effect of Gastric Digestion Prior to Intestinal Digestion on Excystation*

Type of Oocysts	Gastric Digestion, 1 Hr., in	Pancreatic Digestion in	Excystation, per Cent, in 1 Hr.	2 Hr.	8 Hr.
LCF*	Pepsin 1% in 0.5% HCl	Pancreatic juice, dog	0	1	29
LCF*		Pancreatic juice, dog	3	1	28
LCF*	Stomach juice, rabbit	Pancreatic juice, dog	0	1	26
LCF*		Pancreatic juice, dog	0	5	25
LC†	Pepsin 1% in 0.5% HCl	Steapsin, 5%	0	0	30
LC†		Steapsin, 5%	0	1	27
LC†	Stomach juice, rabbit	Steapsin, 5%	0	0	20
LC†		Steapsin, 5%	0	0	23

* Oocysts of Eimeria stiedae collected from the feces.
† Oocysts of Eimeria stiedae collected from lesions of the liver.

The results of these studies are given in table 4. No influence of gastric digestion prior to pancreatic digestion on the excystation of oocysts of Eimeria stiedae obtained from lesions of the liver or collected from the feces could be demonstrated.

EFFECT OF PURIFIED TRYPSIN

It was thought that the combined effects of enzymes other than trypsin in pancreatic preparations, on oocysts, could be eliminated by using commercial preparations of trypsin purified according to North-

rop.[16] Analyses of the enzymatic properties as well as the effects of these purified pancreatic preparations on the excystation of oocysts are presented in table 5.

These analyses show that preparations of pancreas purified from all enzymes but trypsin bring about excystation.

If papain is substituted for trypsin, no effect is obtained. One and five-tenths per cent watery solutions of papain, not activated, as well as activated by 0.1 per cent watery solution of cysteine or by 3 per cent solution of hydrocyanic acid, were adjusted to p_H 5, 6, 7 and 8 by citrate, acetate and phosphate buffers; oocysts from lesions of the liver were then added and the solutions exposed in the incubator to a temperature of 37 C. for eighteen hours. No evidence of excystation could be seen in any of these experiments.

Erepsin.—No evidence of any action of erepsin on excystation of oocysts could be detected. This was proved by several methods.

TABLE 5.—*Analyses of Purified Trypsin and Steapsin*

Medium	Trypsin, Action per Cc. in 20 Min.*	Erepsin, Action per Cc. in 1 Hr.*	Lipase, Action per Cc. in 1 Hr.*	Diastase, Mg. of Maltose Formed per Cc. in 10 Min.	Excystation, % LC;† p_H 7.5; 18 Hr. Exposure
Trypsin, purified.....	0.64	0.0	0.04	0.0	20
	0.62	0.0	0.03	1.0	
Steapsin, purified.....	0.68	0.0	0.01	0.0	34
	0.64	0.0	0.00	0.0	

* Expressed as the number of cubic centimeters of fifth-normal alcoholic potassium hydroxide necessary to neutralize the increase in acidity due to the action of 1 cc. of the enzyme solution on casein (trypsin), leucylglycine (erepsin) or olive oil (lipase) after subtraction of the blank.

† Oocysts of Eimeria stiedae collected from lesions of the liver.

1. The intestinal juice of rabbit P F 6, which had had a fistula of the pancreatic duct for over a week, was collected, centrifugated, and filtered through a Berkefeld filter N. After this fluid had been adjusted to p_H 7.5, oocysts from lesions of the liver were added and the test tubes then put in the incubator at 37 C. for eighteen hours. As control, the effect of the pancreatic juice obtained from the pancreatic fistula of the same animal on the same type of oocysts at the same hydrogen ion concentration and after the same length of time of exposure in an incubator at 37 C. was studied.

No evidence of excystation was found in the samples of intestinal juice of the rabbit with the pancreatic fistula, while the pancreatic juice of this animal gave positive results. The chemical analyses of the two juices yielded the data given in table 6.

2. Negative results were also obtained by the exposure of oocysts to watery alkaline extracts[17] of intestinal mucosa of pigs, as well as to

16. Northrop: J. Gen. Physiol. **6**:340, 1923-1924.

17. Frank and Rice: J. Am. Chem. Soc. **37**:1319, 1915.

watery solutions of erepsin preparations, purified and dried according to the methods described by Euler and Josephson,[18] although these preparations were active when tested chemically.

Lipase.—Experiments with solutions of pancreatic preparations (tables 1 and 5) showed that the presence or the absence of lipase has no influence on excystation.

Amylase.—Although commercial preparations of taka-diastase were found to exhibit a slight effect on excystation, this action was ascribed to the presence of a proteolytic ferment demonstrable in chemical analyses of such preparations (table 1). Besides, experiments with solutions purified of all enzymes but trypsin (table 5) showed that the presence of amylase was not required to accomplish excystation.

From all these experiments it seems highly probable that trypsin represents the factor necessary for the liberation of sporozoites. Further

TABLE 6.—*Comparative Study of Intestinal and Pancreatic Juice of Rabbit with Pancreatic Fistula*

Medium	Trypsin: Activated with Enterokinase, Action per Cc. in 20 Min.*	Trypsin: Not Activated, Action per Cc. in 20 Min.*	Erepsin, Action per Cc. in 1 Hr.*	Lipase, Action per Cc. in 1 Hr.*	Diastase, Mg. of Maltose Formed per Cc. in 10 Min.	Excystation, %, LC;† p_H 7.5; in 18 Hr.
Pancreatic juice, rabbit P F 6....	0.44	0.01	0.0	3.04	120.3	56¶
Intestinal juice, rabbit P F 6.....	0.0	0.0	0.4	0.20	0.0	0¶

* Expressed as the number of cubic centimeters of fifth-normal alcoholic potassium hydroxide necessary to neutralize the increase in acidity due to the action of 1 cc. of the enzyme solution on casein (trypsin), leucylglycine (erepsin) or olive oil (lipase) after subtraction of the blank.

† Oocysts of Eimeria stiedae collected from lesions of the liver.

¶ Effect of pancreatic and intestinal juices activated with enterokinase.

support for this view is given by experiments in which there was demonstrated an increase in the liberating effect of pancreatic juice when the latter was activated by enterokinase. This clearly parallels the effect of activation of trypsin on the digestion of casein.

Commercial preparations of pancreas could not be used for these experiments, because they are usually fully activated. Therefore fresh pancreatic juice was obtained directly from the fistula of a dog and its effect on oocysts tested with and without activation by enterokinase; at the same time, chemical analyses of its tryptic activity with and without activation by enterokinase were performed. These experiments were done in the following manner:

Solution A: To 16 cc. of fresh pancreatic juice 4 cc. of 1 per cent aqueous solution of enterokinase was added and this mixture adjusted to p_H 7.5; 3 cc. of this fluid was put into each of six sterile test tubes, which were then kept in the incubator at 37 C. for one hour before the oocysts were added.

18. Euler and Josephson: Ber. d. deutsch. chem. Gesellsch. **59**:226, 1926.

Solution B: To 16 cc. of fresh pancreatic juice 4 cc. of distilled water was added and the mixture adjusted to p_H 7.5; 3 cc. of this mixture was put into each of six sterile test tubes.

Solution C.: One per cent aqueous solution of enterokinase was filtered through a Berkefeld filter and adjusted to p_H 7.5 by adding drops of 2 per cent solution of sodium carbonate; 3 cc. of this solution was put into each of six sterile test tubes; 3 drops of a suspension of oocysts, either those from lesions of the liver or those from feces, were added to each solution and the test tubes kept in the incubator at 37 C. for seven hours.

The percentages of excystation of the two types of oocysts in the different mediums are given in table 7. The table shows that, although

TABLE 7.—*Influence of Activation of Pancreatic Juice (Dog) on Excystation (p_H 7.5, Seven Hour Exposure)*

Type of Oocysts	Excystation, per Cent		
	Solution A Pancreatic Juice Activated	Solution B Pancreatic Juice Not Activated	Solution C Control, Enterokinase Only
LCF*	25	8	0
LCF*	30	16	0
LC†	25	11	0
LCF*	23	6	0
LCF*	29	12	0
LC†	18	7	0

* Oocysts of Eimeria stiedae collected from the feces.
† Oocysts of Eimeria stiedae collected from lesions of the liver.

TABLE 8.—*Tryptic Action of Pancreatic Juice (Dog) on Casein: Influence of Activation by Enterokinase*

Medium	Duration of Experiment	Tryptic Action per Cc.*	
		Activated	Not Activated
Pancreatic juice, dog	7 hours	1.50 1.52	0.48 0.40

* Expressed as the number of cubic centimeters of fifth-normal alcoholic potassium hydroxide necessary to neutralize the increase in acidity due to the action of 1 cc. of the enzyme solution on casein after subtraction of the blank.

fresh pancreatic juice can act on oocysts without the addition of enterokinase, its action is doubled by activation with enterokinase. Parallel figures are obtained by chemical analyses of the tryptic action of fresh pancreatic juice of the dog on casein with and without activation of the trypsin by enterokinase (table 8).

COMMENT

The excystation of oocysts is a process quite complex and dependent on many factors, which can be divided into two groups: external factors that enable the medium to act on oocysts, and internal factors that rest with the oocysts themselves. The external factors are comparatively easy to control and are represented by the tryptic activity, the

hydrogen ion concentration and the temperature of the medium and the time necessary to effect excystation. The internal factors can be controlled only to some extent and seem to represent the variable in experiments in which the external factors are identical. For instance, oocysts of Eimeria stiedae obtained from lesions in the liver before their natural discharge through the biliary tract and the intestines behave somewhat differently from oocysts of Eimeria stiedae collected from the feces of the same animal. The reason for this difference is obscure; possible causes may be ascribed to: (1) remnants of tissue structure adherent to the shells of oocysts collected from lesions of the liver, which perhaps retard the action of the medium; (2) differences in the character of the sealing material of the micropyle due to immaturity of oocysts collected from the liver, affecting time and speed of excystation.

Slight individual differences of oocysts of the same character are held responsible for the different percentages of excystation in response to different mediums, different hydrogen ion concentrations, and different lengths of time of exposure; owing only to such differences is it possible to express the results in percentages comparable to results of other similar experiments. On the other hand, the individual differences of oocysts supply a large source of errors, because they cannot be calculated. Only marked, fairly constant differences in results can therefore be considered.

In addition to variables of oocysts of the same type, variables of the mediums employed must also be considered, since the tryptic activity of the mediums, measured by the digestion of casein, does not go absolutely parallel with their action on oocysts; the better effect of fresh pancreatic juices on excystation compared with that of juices which have been kept in the icebox for months is out of proportion to the difference in their tryptic action on casein; likewise, the better effect of fresh pancreatic juices compared with the effect of solutions of commercial preparations of trypsin cannot be exactly expressed by the difference in action on casein. Aging as well as alteration of the enzymes due to manipulations during the process of extraction may affect their potency in certain respects.

Although the protein character of the sealing material of the micropyle of oocysts can hardly be questioned, its actual composition is not known; therefore, the comparative studies of the action of trypsin on casein can be only a relative comparative measure of mediums, since the protein of the sealing material of the micropyle certainly is not identical with casein. If the exact nature of this substance were known, a much closer correlation between excystation and the chemical action of a medium could probably be established.

The outcome of the experiments concerning the effect of pancreatic juice and enterokinase indicates that trypsin-kinase (tryptase) is the specific enzyme causing excystation; I am, however, unable to exclude a possible effect of nonactivated trypsin (kinase-free trypsin) on oocysts, because no such preparations were tested in these experiments.

The process of excystation is first induced by the passive destruction or removal of the protein substance closing the micropyle of oocysts; it is, however, completed only by the action of the sporozoites themselves.

It may be assumed that after the opening of the micropyle, the medium exerts a stimulating influence on the sporozoites through the jelly-like matter within the oocysts, which then begin their rotating movements within the sporocyst, and later creep through the micropyle of the sporocyst. During this process the sporozoites take up the shape of an hourglass, the narrowest portion being in the region of the micropyle. Both sporozoites leave their sporocyst successively through the same micropyle; after their liberation, the wall of the sporocyst seems as intact as before, and the opening of the micropyle can no longer be made out. The process of excystation from the sporocyst takes about from ten to twenty seconds. Sometimes only one of the two sporozoites leaves the sporocyst; at other times the sporozoites are apparently unable to escape, and their rotating movements, at first very lively, grow weaker and finally cease altogether. Frequently the sporozoites of only some of the sporocysts attempt excystation, while others show no movements even after prolonged exposure. After having left the sporocyst the sporozoites swim within the oocyst, moving about actively. Immediately they begin to search for the micropyle of the oocyst and try to find it at either pole. After having found the opening, they squeeze themselves through this extremely narrow pore in exactly the same manner as through the micropyle of the sporocyst (fig. 4*B*). The process of leaving the oocyst through the micropyle takes from twenty seconds to several minutes. After the escape of sporozoites from an oocyst, one is unable to see the opening of the micropyle with the oil immersion lens of the microscope even when looking at the top surface of the pole of the oocyst through which the sporozoites have escaped. The site of the micropyle, however, can easily be made out; it is always situated at the flattened end of the oocyst, where the wall of the oocyst is reduced to less than one half of its thickness elsewhere; the micropyle is located in the very center of this thinned out pole, but can be seen only when a sporozoite is creeping through it during the process of excystation. Not all sporozoites succeed in leaving the oocysts. If they cannot escape, their lively movements within the cysts gradually become weaker and then cease. On examining such cysts after prolonged exposure (from twenty-four to forty-eight hours), the structures of the free sporozoites within the oocysts become indistinct, and the organisms gradually degenerate.

In his conclusions, Krijgsman [8] stated that only those oocysts that have completed sporulation are affected by the enzymes; during the sporulation a change in the character of the membranes of the cysts takes place, which makes it then possible for the proteolytic enzymes to act on them. Although it is undoubtedly a fact that such oocysts cannot be affected by proteolytic enzymes, that is, that excystation of incompletely sporulated oocysts cannot be accomplished, the reason for this behavior lies in the imperfect development of the sporozoites during the process of sporulation. Since the excystation is accomplished by the action of the sporozoites themselves, how could they liberate themselves when their development has not been completed?

When the sporozoites have escaped from the oocysts, they move about actively, contracting and twisting their bodies. No flagella are seen when these actively moving liberated sporozoites are examined in the dark-field microscope. These organisms are crescent-shaped and measure from 12 to 14 microns in length and from 2 to 4 microns in width. The anterior tip is pointed, having the shape of a

dolphin's snout. Many refractive granules are seen in the anterior portion of the body, while most of the middle and posterior portion is taken up by a large, homogeneous, shiny substance resembling a fluid droplet. A few refractive granules can also be seen at the pointed tail end of the parasite. In smears kept from drying up by surrounding the coverslip with glycerin jelly, these sporozoites can be easily studied; active movements, gradually becoming weaker, could be observed for more than twenty-four hours after their liberation from oocysts.

CONCLUSIONS

The excystation of oocysts of Eimeria stiedae is induced by the enzymatic action of the pancreatic juice; it is then completed by the spontaneous movements of the sporozoites. The specific enzyme concerned is trypsin-kinase; this can be demonstrated by: (1) the inertness of enzymes other than trypsin as regards influence on excystation; (2) the certain parallelism between the tryptic activity of the medium on casein and the liberating effect of the medium on sporozoites of oocysts, and (3) the influence of activation by enterokinase.

The optimum hydrogen ion concentration for excystation is between p_H 7 and p_H 8.

Excystation takes place at 37 C. and cannot be effected at room temperature.

The length of time required to induce excystation in vitro varies considerably; the minimum is about sixty minutes and the optimum between five and seven hours.

The response to the action of trypsin of oocysts of Eimeria stiedae obtained from lesions of the liver differs somewhat from that of oocysts collected from the feces.

Exposure of oocysts to gastric digestion prior to intestinal digestion does not influence the process of excystation.

LIPOID HISTIOCYTOSIS

REPORT OF A CASE WITH DIAGNOSIS BY BIOPSY

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The diseases included in xanthomatosis, or lipoid histiocytosis, are all considered to be a manifestation of abnormal lipoid metabolism.[1] Rowland [2] and Pick [3] classified the entire group into essential or primary xanthomatoses, which include those that are disease entities in themselves, and secondary or symptomatic, which include those that accompany other diseases. In the former group are included Gaucher's disease, Niemann-Pick's disease, Christian-Schüller's disease, and certain less definite conditions in which there occur localized deposits in one organ. Xanthomatous deposits of the secondary or symptomatic type may accompany diabetes mellitus, chronic hepatic or renal disease and pregnancy. Although the histologic [4] and histochemical [5] changes in the diseases named are different, the etiologic factor common to them all is presumably a disturbance in lipoid metabolism or lipoid excretion. The clinical manifestations of the group depend on the systemic action of the disturbance in metabolism and the localized effects of the deposits.

The diagnoses of these diseases are made by the peculiarities of each condition. In Gaucher's disease, which is ultrachronic, there is hepatosplenomegaly. The typical Gaucher's cells may be found in a smear from splenic puncture. In Niemann-Pick's disease, the course is more rapid, death occurring by the third year, and there are generalized adenopathy and hepatosplenomegaly. Again characteristic lipoid-laden cells may be seen in smears from bone or splenic puncture. Christian-Schüller's disease is characterized by storage of lipoid in membranous bones (chiefly), with localized symptoms of diabetes insipidus and exophthalmos.

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REPORT OF CASE

A child, 2 years of age, was referred to Pondville Hospital (by Dr. Charles Dumas) with a tentative diagnosis of lymphoblastoma (Hodgkin's type). She was of Italian parentage. For five months she had had bilateral painless cervical lymphadenopathy. Six months prior to the onset of the cervical adenopathy she had had acute tonsillitis lasting two weeks. No swelling in the neck was noted at that time. The past history including the birth history was without significance. She was the first child, a normal delivery, from healthy parents. She was breast-fed and had had no previous illnesses except tonsillitis. One brother, 5 months old, was living and well. The paternal grandmother had tuberculous adenitis. The rest of the family history was essentially negative.

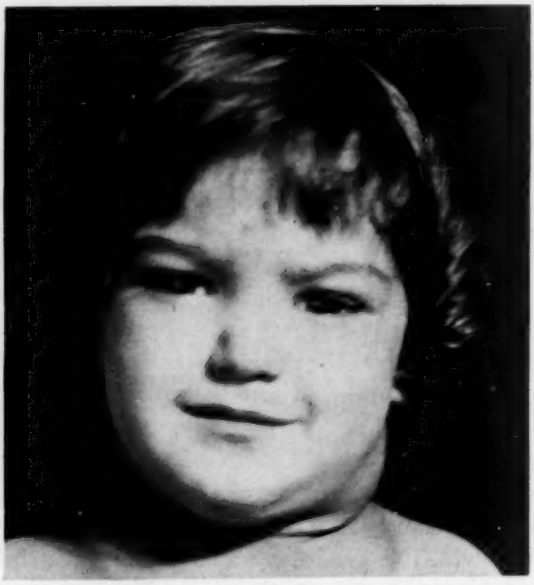

Fig. 1.—Photograph of child after her admission to Pondville Hospital.

The physical examination revealed a well developed and slightly undernourished, mentally alert child of 2 years, weighing 23¼ pounds (10.4 Kg.). She had bilateral masses averaging 4 by 3 by 3 cm. in both anterior and posterior triangles of the neck. There was a biopsy scar over one of these. The nodes were elastic and not adherent to the skin or overlying tissue. The head, heart and lungs were normal, and neither the spleen nor the liver nor masses were palpable in the abdomen. There was no evidence of rickets or of nutritional disease. No other findings were noted. The temperature, pulse and respiration were normal. The urine was normal. The red blood cells numbered 4,310,000, with considerable poikilocytosis and anisocytosis. The leukocyte count was 13,900, with a differential count of 75 per cent neutrophils, 4 per cent eosinophils, 15 per cent large lymphocytes and 6 per cent monocytes. The platelets were normal. The Wassermann reaction was negative.

Because the admission diagnosis of lymphoblastoma (scirrhous type) was based on a biopsy done elsewhere, and because deep roentgen treatments had been given

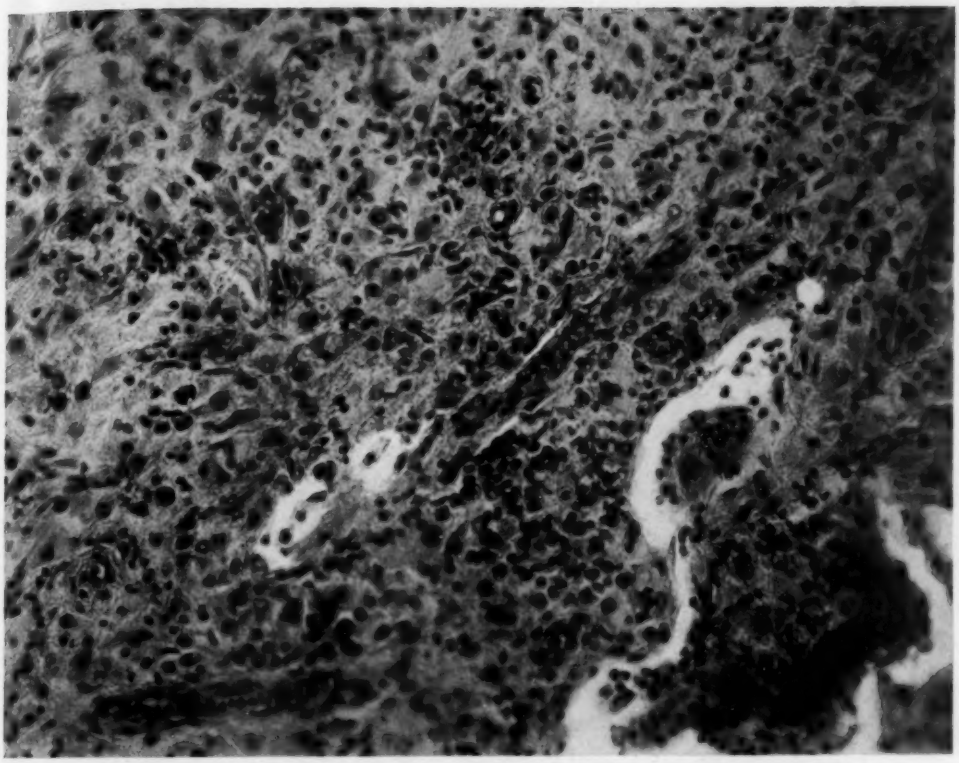

Fig. 2.—Photomicrograph of cervical lymph node showing infiltration with lipoid-laden cells; × 325.

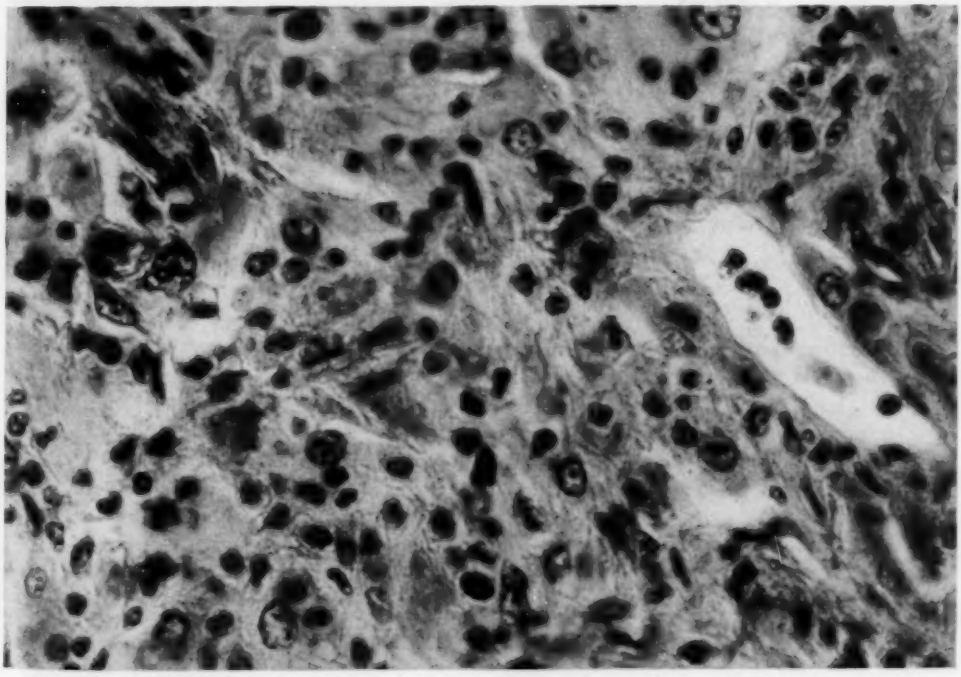

Fig. 3.—Higher power view of figure 2. Note vacuolization of cells and mitosis in histiocyte; × 700.

with no noticeable decrease in the size of the nodes in the neck, another biopsy from the cervical nodes was done (by Dr. E. M. Daland) at Pondville Hospital before treatment.

The specimen consisted of two enlarged, moderately soft lymph nodes averaging 3.5 by 1 by 1 cm. in diameter. They were encapsulated and cut with decreased resistance, revealing a smooth, grayish-pink, glassy surface mottled with streaks of grayish yellow.

Microscopically, the structure of the node was considerably distorted. Only a few secondary nodules persisted, and these were small. The bulk of the node was occupied by large cells ranging up to 40 microns in diameter. These cells had

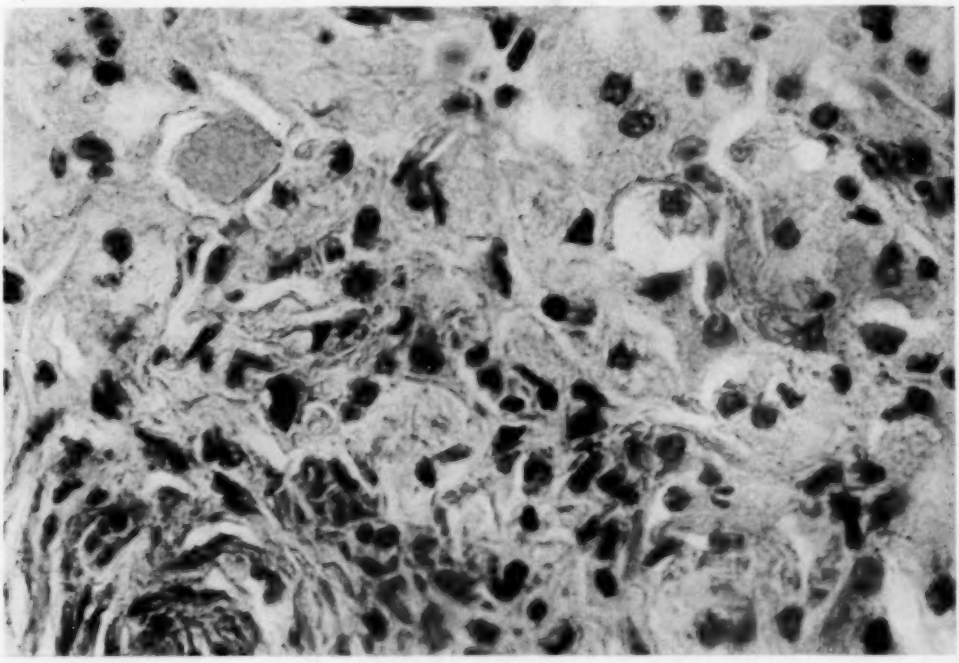

Fig. 4.—High power view of figure 2. Note pale vacuolated histiocytes; × 700.

a well defined, round to ovoid, vesicular nucleus with one prominent nucleolus. Very occasional mitotic figures were seen. The cytoplasm was large in amount, ranging in staining reaction from faintly basophilic to markedly acidophilic. The cytoplasm varied in texture from coarsely vacuolated or foamy to almost homogeneous. In a few cells, large vacuoles occupying almost the entire cell were found. The cell outlines were fairly well defined.

In general, adjacent groups of these cells showed similar changes, so that the section had a slightly mottled appearance due to the variation between heavily vacuolated cells and those showing slight vacuolization. The sinusoids contained small numbers of these cells, but the endothelium of the sinusoids was apparently normal. In a few foci there was a slight amount of fibrosis. The capsule of the node was intact.

Frozen formaldehyde-fixed sections stained with sudan IV showed heavy red-staining of these vacuoles. Paraffin sections fixed in Zenker's solution and stained with Mallory's aniline blue showed a bluish tinge to these vacuoles.

Sections of a second lymph node showed a less advanced condition. Here there was less distortion of the structure of the node, and the vacuolated cells were seen to be distributed chiefly about the sinusoids. This section also showed moderate infiltration by eosinophils of both lymphoid tissue and tissue made up of these vacuolated cells. There was slight perisinusoidal fibrosis in this section also. In a few foci, partial fibrosis and hyalinization of the secondary nodules had occurred. Rarely, the large vacuolated cells had fused to form giant cells with six or eight discrete nuclei without definite arrangement.

The microscopic diagnosis was lipoid histiocytosis.

After the diagnosis of lipoid histiocytosis had been made, the following additional laboratory data were gathered. The blood sugar was found to be 160 mg. per hundred cubic centimeters of blood; the blood serum cholesterol, 148 mg.; the blood serum fatty acids, 270 mg., and the total serum lipoids, 430 mg. Roentgenograms of the chest and entire skeleton revealed no abnormalities.

The patient received 600 roentgen units of deep x-rays on the right and left sides of the neck. A questionable decrease in the size of the cervical masses was noted. After she had been in the hospital a month, palpable nodes were found in the right groin, and subsequently 400 roentgen units of deep x-rays were given. She was discharged six weeks after admission with no improvement in the size of the nodes but otherwise well. The mother was advised to place her on a low fat, high carbohydrate diet. She has been seen twice during the past six months after her discharge from the hospital, looking well and with no complaints, although showing the same involvement of the lymph nodes.

COMMENT

This case exemplifies the essential or primary lipoid histiocytosis wherein lipoid is deposited in an organ or localized part of the body—here, the lymph nodes. The diagnosis of lipoid histiocytosis by biopsy of a lymph node has not been reported in the literature.[6] Gaucher's, Niemann-Pick's and Christian-Schüller's diseases cannot be considered here, because of the absence of an enlarged spleen or liver, the fairly good general health and the negative results of roentgen examinations of the skeleton. Although the lipoidal elements of the blood are within normal limits in the case reported, as Rowland pointed out, the blood serum lipoid may be either increased, normal or decreased, as the disease is probably a faulty storage of the lipoid.

6. We were singularly fortunate in that we were able to show the slides of the lymph node to Dr. Ludwig Pick during his recent visit to Boston. He concurred in the diagnosis, as did Drs. F. B. Mallory and R. S. Rowland.

MOUSE LEUKEMIA

VIII. CONTINUITY OF CELL LINEAGE IN TRANSMISSION LINES OF LYMPHATIC LEUKEMIA

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Since the first experimental transfer of lymphatic leukemia in mice in 1929,[1] fourteen transmission lines have been established in these laboratories and carried for numerous transfers. Each line has developed certain characteristics which remain relatively constant in successive transfers, among which are the interval between inoculation and death of the host and considerable uniformity in the gross and microscopic pictures at autopsy.

A specificity of the susceptibility of certain strains of mice to certain lines of leukemia [2] has been shown to be under genetic control. A genetic analysis of the susceptibility of mice of strain C58 to line I has been reported.[3]

To the present time all efforts have failed to produce from leukemic tissue of mice a cell-free filtrate which will cause leukemia when introduced into a new host.[4] This is also true of the guinea-pig,[5] though fowl leukemia has been successfully transmitted after filtration.[6] But the question of the origin of the infiltrating cells in successively inoculated mice, whether they arise from cells introduced at the time of inoculation of the hosts, or whether they arise from existing tissues in the host which have been stimulated by an introduced agent, has been

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The histologic and cytologic material was collected and prepared with the assistance of Miss Margaret D. Findley.

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a matter of conjecture. As an approach to this problem, the existence of a predominating cell type in the lesions caused by the injection of line I has been reported and the cell described.[7] The present paper gives the results of cytologic and histologic investigations on three transmission lines which provide direct evidence on this question.

METHODS AND MATERIALS

Methods of Inoculation.—Routine inoculations were made by mincing a spleen obtained from a host of the previous transfer generation, in 0.9 per cent saline solution and injecting 0.2 cc. of the suspension intraperitoneally into each new host. In order to restrict the dissemination of the inoculum, which is not possible with intraperitoneal inoculations, the suspension was also inoculated in the thigh muscles of the right hind leg in another group of animals. The latter technic promised to facilitate the identification of the injected cells in the earliest stages following inoculation. The transfer generations of the lines used, the number of animals involved and the sites of inoculation are summarized in table 1.

TABLE 1.—*Animals, Lines of Leukemia and Sites of Inoculation Used*

Strain of Mice	Line of Leukemic Cells	Transfer Generation	Number of Animals	Site of Inoculation
C58	I	122, 123, 126, 134	92	Intraperitoneal
C58	I	134, 152	20	Leg
C58	MsplB	3	15	Leg
C58	MsplC	3	4	Intraperitoneal
89	L	34	15	Intraperitoneal
C58	Uninoculated	...	20	
89	Uninoculated	...	6	

Lines Used.—Three contrasting transmission lines were selected for these studies on the basis of their easily observed differences.

At the beginning of these studies, line I had been carried for 2½ years by a series of 124 young mice of strain C58, the strain in which the spontaneous case occurred from which line I originated. The survival of the hosts after inoculation was less than a week.

Line L originated from a spontaneous case in strain 89 and is carried in young mice of that strain. It was in its thirty-fourth transfer generation, and the hosts survived slightly more than a week.

Line MsplB originated from a spontaneous case in strain C58, and is carried in young mice of that strain. During the third transfer, when the line was used for these studies, the period of survival of the hosts averaged 15 days.

Gross autopsies showed that there was a characteristic distribution of lesions in the animals inoculated with each of these lines.

Material Used.—Since 100 per cent susceptibility of hosts was assured by using certain strains of mice as hosts for certain transmission lines, and since it was known that there was a characteristic distribution of lesions and a characteristic interval between inoculation and death with each of the lines, it was possible to obtain tissues representing regular steps in the development of the leukemic condition by killing animals at regular intervals following the inoculation. Two animals were killed from each series at the end of each 24 hour period following

7. Potter, J. S., and Richter, M. N.: Studies in Mouse Leukemia: VI. The Predominating Cell Type in Line I, Proc. Nat. Acad. Sc. **18**:298, 1932.

inoculation until death. In addition two animals were killed at each 4 hour period during the first 24 hours of the hundred and thirty-fourth transfer generation of line I.

Control animals of the same age and strain as the hosts and kept under the same environmental conditions were killed and examined during each experiment. This provided a comparison of the activity of lymphoid tissues of uninoculated and inoculated animals.

The following tissues and organs were always removed and fixed in either Winge's picro-Carnoy B15 or Flemming's fluid: cervical, axillary, thoracic, mesenteric, pyloric, renal, lumbar and inguinal lymph nodes; spleen, thymus, lungs, liver, pancreas, kidneys, genitals and abdominal wall. Any other tissue which showed possible infiltration at autopsy were also fixed. Material from terminal stages fixed in Helly's fluid was also available.

Organs were individually embedded in paraffin and completely sectioned serially at from 5 to 8 microns. Flemming's triple stain, Heidenhain's iron-alum hematoxylin, or hematoxylin-eosin were used for routine staining. For specific cytologic details, certain special methods were employed.

Flemming's strong solution or Hance's modification at from 3 to 5 C. for twelve hours gave good fixation of small pieces of tissues for chromosomes. Dividing cells in the peripheral blood were observed after fixation of blood smears in Flemming's weak solution followed by Wright's stain or by gentian violet.

For mitochondria, both fixed and supravital technics were used, Murray's method for fixed material and Janus green as a supravital stain. Golgi's apparatus in the infiltrating lymphocytes was studied after the Mann-Kopsch[8] method of osmic impregnation. The chromatin and nucleolar content of the nuclei could be studied after any suitable fixation and staining, but the Feulgen technic gave more specific results.

Smears of peripheral blood were made at intervals and stained either with Wright's stain or with Wright-Giemsa stain.

PREDOMINATING CELL TYPES IN THE LINES STUDIED

In order to trace the inoculated cells with accuracy after inoculation of hosts with different lines it was necessary to study in detail the cell types occurring in each line. The lesions that followed inoculation were studied in all stages of development and the predominating types for the various lines are described in the following paragraphs. The name "type cell" will be used to designate the predominating cell of the particular line being discussed. For purposes of comparison, the description of the type cell of line I previously published[9] is repeated here with additional details.

Size and Shape of Cells.—The lesions formed after inoculation of a host with line I are characterized by accumulations of cells measuring from 9 to 11 microns in fixed preparations. The irregular shape of the cell is characteristic (fig. 1). The size of the cells in lesions following inoculations of line L and line MsplB is smaller, falling between 6 and

8. Lee, B.: Microtomist's Vade Mecum, ed. 9, Philadelphia, P. Blakiston's Son & Co., 1928.

9. Potter, J. S., and Richter, M. N.: Proc. Nat. Acad. Sc. **18**:298, 1932.

8 microns, and the shape of these cells is always more nearly rounded. Distributions of individual measurements are given in table 2.

Measurements were made with a Bausch and Lomb movable scale micrometer eyepiece calibrated against a stage micrometer. The starred series in the table were made by selecting ten cells in each microscopic field of measurement. The other four series are composed of measurements of all the rounded-up cells in the microscopic field.

Cytoplasm.—In smear preparations stained with Wright-Giemsa stain, most of the I type cells show a distinct basophilic reaction of the abundant cytoplasm. Azure granules are few or absent. There is less

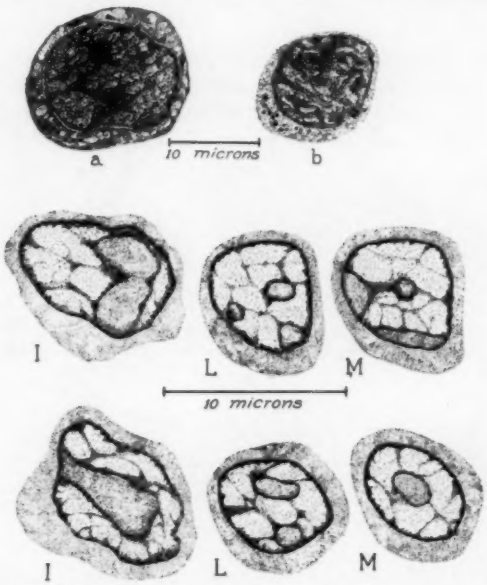


Fig. 1.—Type cells of lines used: (*a*) line I type from peripheral blood; (*b*) line L type (*a* and *b* from dry films, Wright's stain); (*I*) line I type from infiltrated tissue; (*L*) L type; (*M*) MsplB type (*I*, *L* and *M*, camera lucida outlines from sectioned material).

cytoplasm in the L and MsplB type cells; the basophilic reaction is weaker than in the I type, while azure granules are always present.

There are more mitochondria in the cytoplasm of the I type cell than in that of the L cell. A summary of counts made in supravital Janus green preparations is given in table 3.

The Golgi apparatus in the type cells of all three lines may be demonstrated readily after Mann-Kopsch fixation as a group of connected loops and rods. Lymphocytes from normal uninoculated mice have the same type of net, and by inspection (measurements being impossible) there appears to be no constancy in size or in peculiarity in shape for a given type cell.

Nucleus.—The nucleus of the I type cell is usually very irregular in shape as compared with the more rounded nuclei of the L and MsplB types. The chromatin is arranged in an open network with accumulations about the nucleolus and nuclear membrane of greater density in the I type than in the other types.

Usually only one large plasmasome nucleolus is present in the I type cell. The plasmasome is elongated and often lies adherent to the nuclear membrane through most of its length. From one to four smaller plasmasomes are distributed through the nuclei of the L and MsplB type cells. There is a measurable difference in the nuclear arrangement between the I and L type cells: the mean values for per

TABLE 2.—*Measurements of Infiltrating Cells in Different Lines*

Line	Transfer Generation	Number of Cells of Given Diameter in Microns					
		5+	6+	7+	8+	9+	10+
I	134	..	..	5	10	38	47
I	134	..	..	..	2	63	25
I*	152	..	..	..	1	54	37
L*	27	1	14	36	2	..	..
L	34	4	22	80	..	..	..
L	34	3	44	55	9	..	..
MsplB	3	..	34	63	3	..	..

* The starred series of counts was made by selecting 10 cells in each microscopic field for measurement. The other four series are based on measurements of all the rounded-up cells in the microscopic field.

TABLE 3.—*Number of Mitochondria in Type Cells of Lines I and L*

Line	Number of Type Cells Showing Given Number of Mitochondria			
	±5	±10	±20	30+
I	..	1	18	134
L	..	100	36	14

cent of the area of greatest optical cross-section of the nucleus occupied by the greatest area of plasmasome (areas obtained by measuring camera lucida outlines with a planimeter) show a significant difference. The mean for line L (137 cells) was 12.39 ± 0.41 per cent; for Line I (136 cells), 22.70 ± 0.39 per cent; the difference is 10.31 ± 0.56 per cent.

Chromosome counts were made during the late prophase of divisions in sectioned material of line I. The smaller size of the dividing cells in the other lines prevents accurate counts in sectioned material. However, the observed bipolarity of the mitotic spindle, the regularity of chromosome movement during the anaphase, and the regularity of other stages do not point to an anomalous division. Although dividing cells were not seen in the peripheral blood of animals inoculated with line L, they were abundant in the peripheral blood of the hosts of line I

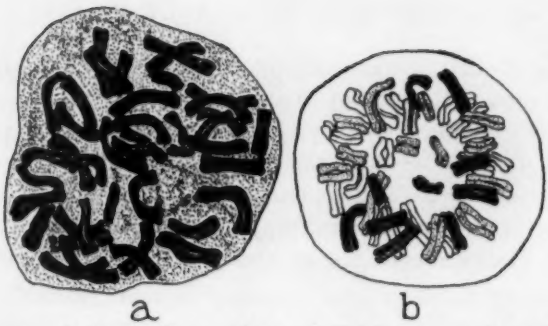


Fig. 2.—Camera lucida outline analyses of division stages in peripheral blood; approximately × 3,000. The drawings are made from films in Flemming's fluid and stained with gentian violet: (*a*) late prophase, line I; (*b*) metaphase, line MsplB.

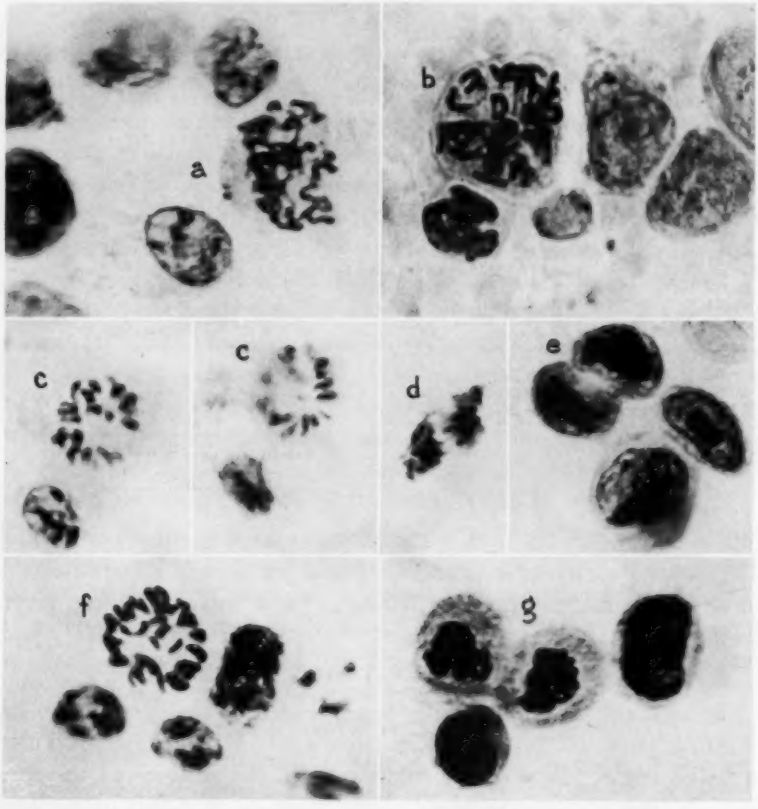


Fig. 3.—Mitosis of leukemic cells in the peripheral blood; × 1,250; from blood films fixed in Flemming's fluid and stained with gentian violet: (*a*) spireme; (*b*) prophase chromosomes; (*c*) metaphase showing clearly the splitting of the chromosomes which has occurred during the prophases, but is not apparent in the preceding photomicrograph; (*d*) anaphase; (*e*) telophase (*a-e*, line *I*); (*f*) metaphase; (*g*) telophase (*f* and *g*, line *M*).

and line MsplB. By the use of blood films fixed in Flemming's solution and stained with gentian violet, the diploid nature (40 chromosomes) of the leukemic cell may readily be demonstrated (fig. 2). The precocity of chromosome-splitting previously reported [8] is seen in such preparations (fig. 3). The chromosomes are distinctly double before the metaphase. A precocious splitting of chromosomes in cells of malignant growth has been reported by Lewis.[10]

Frequency of Division.—By counting the total number of infiltrating cells in a given microscopic field and the number in division, a measure of the frequency of division in a given line may be obtained. Such counts were made at various stages in development of the lesions and

TABLE 4.—*Percentages of Cells in Division*

Line I				Line L				Line MsplB			
Sample	Number of Cells	Number Dividing	Per Cent Dividing	Sample	Number of Cells	Number Dividing	Per Cent Dividing	Sample	Number of Cells	Number Dividing	Per Cent Dividing
1	137	8	5.83	1	107	5	4.66	1	414	11	2.56
2	94	5	5.31	2	143	5	3.49	2	128	3	2.34
3	64	5	7.81	3	145	8	5.51	3	252	6	2.38
4	209	17	8.13	4	170	6	3.52	4	134	2	1.49
5	183	15	8.19	5	307	14	4.56	5	224	4	1.78
6	159	10	6.28	6	254	8	3.14	6	502	10	1.99
7	131	10	7.63	7	345	9	2.60	7	217	4	1.84
8	167	9	5.39	8	380	11	2.89	8	182	3	1.64
9	83	5	6.02	9	175	4	2.28	9	122	1	0.81
10	164	7	4.26	10	413	21	5.08	10	138	3	2.17
11	128	11	8.59	11	251	9	3.58	11	79	1	1.26

Summary				Comparison		
						Difference
Line	Number of Cells	Number Dividing	Per Cent Dividing	Lines Compared	Difference in Percentages	Standard Error
I	1,519	102	6.71 ± 0.69	I-L	3.00 ± 0.74	4.1
L	2,690	100	3.71 ± 0.37	I-MsplB	4.70 ± 0.70	6.7
MsplB	2,389	48	2.01 ± 0.29	L-MsplB	1.70 ± 0.47	3.6

in various parts of the host. The division stages counted were limited to late prophases, metaphase and anaphase for ease of recognition. The results of the counts made in lesions of the three lines are given in table 4.

DAILY PROGRESS FOLLOWING INOCULATION

The type cell of a line having been identified, the progressive stages of infiltration could be studied in tissues taken from animals killed at successive stages following inoculation. All the sections of the serially sectioned organs were examined. The daily distribution of infiltration is plotted diagrammatically in figure 4.

Line I, Intraperitoneal Inoculation (Fig. 4, Second Series).—In order to observe the position of the inoculum after intraperitoneal

10. Lewis, Margaret R.: Abstr., Anat. Rec. (supp.) **52**:65, 1932.

inoculation, animals were killed immediately after the injection. The mass of the injected material was found to lie in the anterior part of the abdominal cavity near the spleen. Examination of sections from animals killed 4 hours after the inoculation substantiated the gross observations; the mass of the recognizably injected cells was lying between the pancreas and the spleen, with smaller accumulations near the mesenteric node, and in the region of the lumbar nodes and the genitals. These early sections showed a typical reaction to irritation on the part of the host, with extensive migration of polymorphonuclears to the area occupied by the injected cells. The injected mass contained degenerating erythrocytes and other cells included in the suspension, as well as active type cells. During the first 12 hours many of the cells in the center of the injected mass degenerated, while cells in the periphery of the mass were dividing and starting to invade the immediately surrounding tissues.

During the second 12 hour period, the mass originally injected greatly increased in size. At the end of this period, small perivascular accumulations were visible in the liver, and in the mesenteric and lumbar nodes small infiltrations were evident.

At the end of 48 hours, the accumulations had increased markedly in number and size. The lumbar nodes were heavily infiltrated, and small accumulations of the I type cell were visible throughout the red pulp of the spleen. The infiltrations in the liver had become more general, with enlargement of the earlier infiltrations due to cell division as well as to new arrivals. Small areas of infiltration appeared in the lungs in a perivascular position. The cervical nodes showed the first definite appearance of infiltration, and in the abdominal wall infiltrations extended along the fascial planes, spreading the bundles of muscle fibers.

The tissues taken at 72 hours showed enlarged accumulations in all previously affected areas. In addition, the axillary and thoracic nodes, the kidneys and the genitals (excepting the testes) had been infiltrated. By 96 hours, the infiltrations of the organs had become massive, and the normal architecture of the lymphoid tissues was obliterated. Degeneration had commenced in parts of some lesions. This was further emphasized in animals killed after 120 hours. Death of host animals occurred usually between 120 and 144 hours.

Line L, Intraperitoneal Inoculation (Fig. 4, First Series).—The stages immediately following intraperitoneal inoculation with line L showed the establishment of the inoculum in the same areas as in the case of line I, with most of the cells in the periphery of the mass surviving and many of those in the interior of the mass degenerating. The host response gave a typical picture of irritation.

Unlike line I, line L presented no decided change during the second 24 hour period. Material taken at 72 hours showed considerable

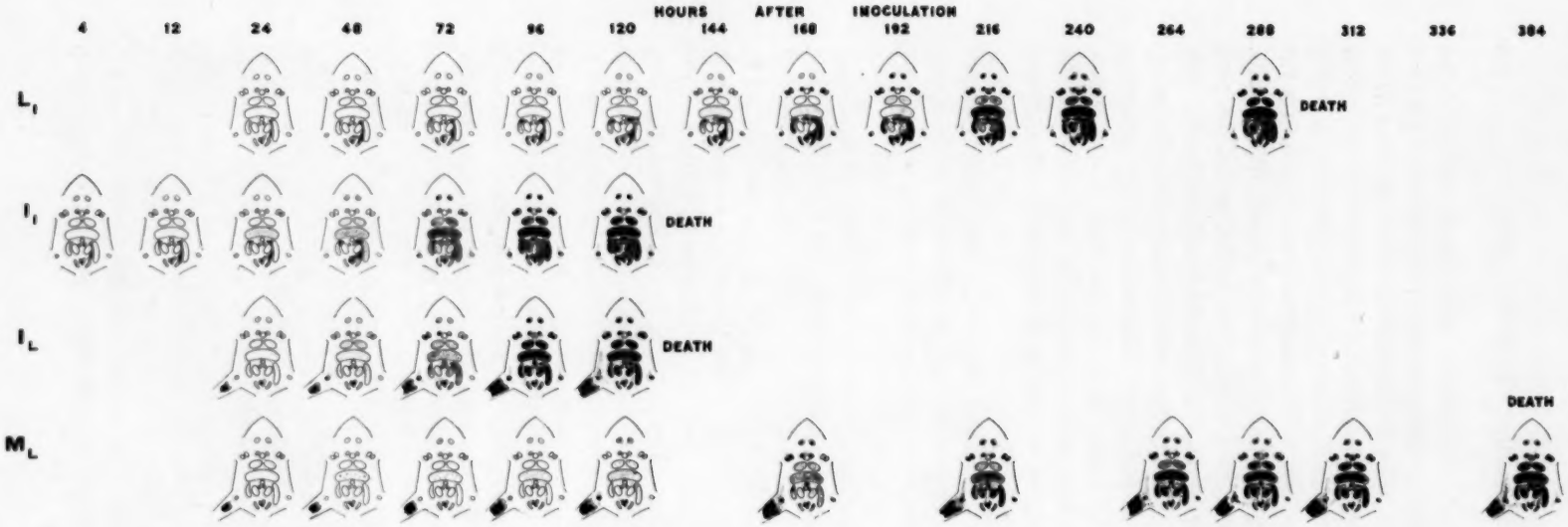
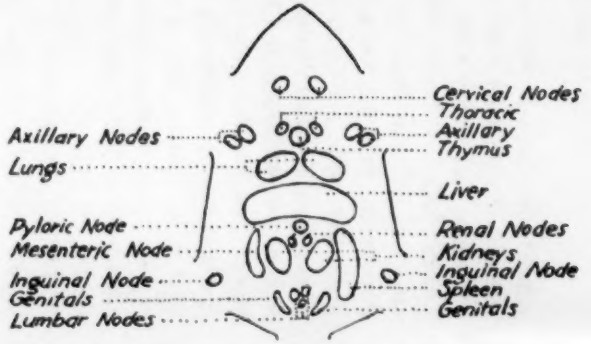


Fig. 4.—A diagrammatic representation of the daily progression of inoculated type cells of lines L and I, after intraperitoneal injection (L_I and I_I) and after injection into the leg (I_L and M_L). A key to the diagram is shown at the right.



enlargement of the injected mass, as well as the first infiltration of the mesenteric nodes and the wall of the uterus. The only change at 96 hours compared with the preceding 24 hour period was an enlargement of the existing accumulations. The earliest splenic invasion was observed at 120 hours, followed at 144 hours by perivascular accumulations in the liver and further invasion of the mesenteric and lumbar nodes and the genitals.

A definite change appeared after 168 hours. Infiltrations appeared in the thoracic, axillary, inguinal and cervical nodes, as well as a great increase in the extent of previous invasions. Infiltrations appeared in the kidneys, lungs and thymus at 192 hours, followed by a great increase in the size of the lesions and degeneration in parts of the older lesions until at 288 hours maximum infiltrations were observed, although in some cases death of hosts occurred as early as 216 hours.

Line I, Leg Inoculation (Fig. 4, Third Series).—By injection into the thigh muscles, the inoculated material was held in position in one mass. Only 0.1 cc. of the suspension was used. Degeneration of part of the injected mass occurred at once, with accumulations of polymorphonuclears as part of the host's response to the irritation. Here, as in the other types of inoculation, the cells in the outer portion of the injected mass soon began to divide. The infiltration commenced by progression along the fascial planes, which resulted in the spreading of the bundles of muscle fibers. During the first six 4 hour periods there was a steady increase of the original mass, but nodal or organic infiltrations were absent. At the end of 24 hours, the node in the right side of the lumbar group showed infiltrations by I type cells in the peripheral sinus and in the cortex. No other infiltrations were found in any of the sections of the organs at this time.

By 48 hours the mass in the leg had increased greatly; the lumbar nodes were heavily infiltrated, while the right inguinal node was only slightly infiltrated. Small perivascular accumulations appeared in the liver and left kidney and I type cells were scattered through the lungs. Other tissues showed no invasion.

By 72 hours the mass in the leg had grown to about ten times the original size, and the lumbar nodes were heavily infiltrated. The accumulations in the liver and lungs had increased in number and size, while both kidneys showed small perivascular accumulations. The renal nodes were infiltrated as well as the loose connective tissue surrounding the nodes. This was also true of both inguinal nodes. The right axillary nodes were heavily infiltrated, while the left axillary showed no infiltration. The thoracic nodes were infiltrated unequally. There were subcapsular accumulations in the epididymis of one male, and both animals showed infiltrations of the connective tissues around the genitals. The mesenteric node was moderately infiltrated as was also the spleen.

Infiltrations reached the maximum extent observed at 96 hours, both in lymphoid tissue and other organs. The larger lesions (especially in the leg and lumbar regions) showed some degeneration. Animals surviving until 120 hours showed the same picture as those examined at 96 hours, except for an increase in the amount of degeneration.

Line MsplB, Leg Inoculation (Fig. 4, Fourth Series).—The same technic was used in inoculating a series with MsplB as was used in the series inoculated with I. The initial response of the host was the same: Polymorphonuclears collected around the injected mass, and the center of the mass showed considerable degeneration during the first 24 hours. The peripheral cells survived in large numbers and commenced dividing and passing out along the fascial planes.

No infiltrations were found, however, outside of the enlarging inoculated area until 48 hours, when a small number of type cells appeared in the right node of the lumbar group, and a few small accumulations appeared in the liver. Animals killed at 72 hours showed essentially the same picture but for an increase in the size of existing accumulations.

At 96 hours, the right inguinal region showed suffused lymphatics and small accumulations in the connective tissues surrounding the node and in the peripheral sinus and cortex of the inguinal node. By 120 hours, the lungs and the spleen had become slightly infiltrated, while the size of the previously existing accumulations, especially at the site of inoculation, had increased greatly. A slight invasion of the cervical nodes was observed.

After 168 hours had elapsed, all the nodes showed varying degrees of infiltration, the kidneys and the genitals were invaded, and a general increase in the size of the older invaded areas was apparent. From this point on, until 288 hours, the lesions increased greatly in size. From 288 hours until the first death (384 hours) many of the lesions showed large areas of degeneration.

MANNER OF TYPE CELL DISSEMINATION

The distribution of lesions at successive stages in the four series, summarized diagrammatically in figure 4, indicates a similarity of path of dissemination of cells, starting from a given site of inoculation. The changes appearing in the initial stages depend on the motility of the leukemic cells themselves. This is shown most effectively by inoculation in the leg, after which the area of infiltration about the injected mass is observed to increase by the migration of individual cells along the fascial planes (fig. 5*a*). This same type of ameboid locomotion and active penetration is shown in the abdominal wall after intraperitoneal inoculation with line I (fig. 5*b*), and in the invasion of the spleen directly through the splenic capsule by type cells of line L.

In the latter case, the proliferation of cells in the injected mass has been observed adjacent to the spleen in the hosts killed during the first 4 days, without infiltration of the spleen itself. On the fifth day the sections of the spleen of one of the animals killed showed entrance of

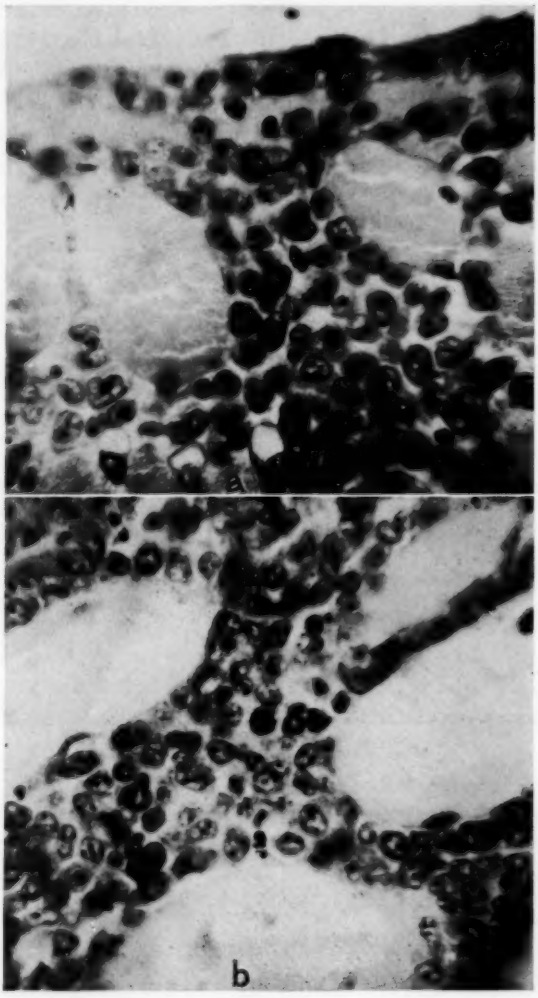


Fig. 5.—Infiltration along fascial planes: (*a*) type cells of line I infiltrating the abdominal wall; (*b*) type cells of line MsplB infiltrating the leg above the site of inoculation.

cells from the injected mass directly through the fibers of a limited area of the splenic capsule into the spleen. Whether an injury to the capsule had occurred is not known, but a considerable penetrating power on the part of the cells is indicated.

The wider distribution of the type cells and the formation of lesions at a distance from the injected mass are probably consequent on transportation through the lymphatics, which become suffused with the type cells near the injected mass. After this, the order of appearance of accumulations in different lymph nodes depends on the site of inoculation. Lesions are widely distributed before the blood becomes leukemic. This is shown by comparison of daily peripheral blood counts (fig. 6) for the line I and line L series, and the accompanying spleen lengths, with the daily distribution charts (fig. 4).

The manner of entrance into the lymph nodes is a further indication of the activity of the lymphatics in the dissemination of injected cells and their offspring: before or simultaneously with the infiltration of a

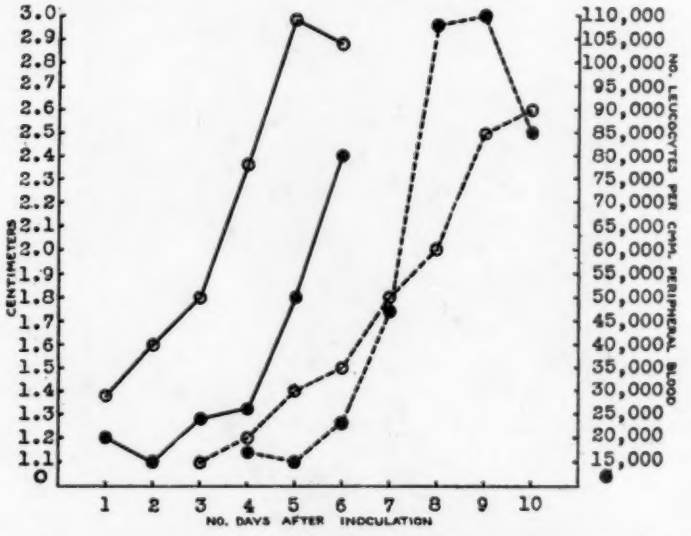


Fig. 6.—Daily average leukocyte counts in peripheral blood and spleen lengths of two series of mice, one inoculated with line I and one with line L. The solid lines represent the spleen lengths (open circles) and the leukocyte counts (solid circles) of the mice inoculated with line I. The broken lines represent the spleen lengths (open circles) and the leukocyte counts (solid circles) of the mice inoculated with line L.

node, type cells appear in the lymphatics entering the node, the peripheral sinus becomes suffused, and a general distribution through the nodal tissue follows.

LYMPHOID TISSUE OF HOST

Because of the possibility of a stimulating agent increasing the production by host tissues of lymphocytes morphologically the same as type cells of the line used, special attention was paid to the lymphoid tissues in hosts as compared with the same tissues in uninoculated animals of

the same age and strain, kept under the same environmental conditions and killed during the course of the experiments. The results of this comparison showed that the numbers of germinal centers were approximately the same in the inoculated and uninoculated groups, and that the germinal centers were not more active in the inoculated animals.

As the nodal and splenic infiltrations became greater in inoculated animals, the normal architecture of the lymphoid tissue was destroyed until type cells of the line injected predominated. The proliferation of the cells was not limited to definite areas, as occurs in normal nodes, but occurred in all parts of the infiltrated area in the same manner as in the injected mass.

It so happened that in both types of inoculation used the injected mass lay near to a host lymph node. This offered a particularly good chance to study the response of the nodal tissue to inoculation, particularly with lines L and MsplB, because of the slower progress of these two lines in infiltrating. In no case was a change noted in the nodes of the daily series until infiltration by the line type cells began in the manner previously described.

Because of the absence of cells intermediate between connective tissue cells (from which lymphocytes have been derived by some) and the leukemic cells, there is no evidence of local transformation of host cells into type cells in the infiltrations.

SUMMARY AND CONCLUSIONS

A cytologic description is given of the cell types predominating in lesions resulting from the inoculation of hosts with line I, line L and line MsplB of transmissible lymphatic leukemia of mice. The cell type for a given line is shown to remain constant for numerous transfers in size range, characteristic shape, average frequency of division, distribution of number of mitochondria and nuclear-plasmasome ratio. The type of Golgi's apparatus is the same for all type cells, and the chromosome number is always forty, the normal complement for the mouse. There is a precocious splitting of the chromosomes during division, which has been reported as characteristic of certain malignant growths. The leukemic cells undergo but slight differentiation, only division, a condition similar to that found in tumors.

The daily progress of infiltration by the type cells of the three lines is traced from the injection until the death of the host. Each line of cells is shown to have a characteristic rate of penetration, and the injected cells or their descendants are shown to be responsible for the formation of lesions characteristic of the line. The lymphoid tissues of the hosts are not stimulated in the formation of lymphocytes as a result of inoculation.

Since there is no increase in the production of lymphoid cells by the hosts, either in lymphoid tissues or locally from connective tissues, and since the injected cells or their descendants may be traced to infiltration and formation of lesions in the host tissues, the conclusion is reached that the infiltrating cells of a given line of transmissible lymphatic leukemia of mice are the descendants of the cells of the original spontaneous case.

A SIMPLE QUANTITATIVE MICROCRYSTALLOGRAPHIC ESTIMATION OF PHOSPHATES IN URINE

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Microcrystallographic methods have been used for microchemical analyses, for the differentiation of isomeric compounds and also for the identification of small quantities of elements (Curtmann,[1] Billmann [2]). This article will demonstrate that a microcrystallographic reaction can be used for a quantitative estimation of the phosphates in the urine. The method is based on the extensive clinical observations of J. Henry Dowd (Buffalo).[3] If urine is precipitated with an ammonium magnesium sulphate reagent of a certain concentration (Dowd's reagent), the volume of the precipitated crystals, their form, and the velocity with which they develop vary according to the amount of phosphates in the urine (Clemesha [4]).

The article presented here will be limited to the chemical foundation of the method and to the technic of an exact quantitative application. Further articles will deal with the results of its practical use for clinical purposes.

THE CHEMICAL FOUNDATION

In order to determine the relation between the form of the crystals and the amount of phosphorus in a solution, increasing quantities of the following solutions were used: neutral sodium phosphate, disodium phosphate (the "secondary" salt), monosodiumphosphate (the "primary" salt), and the orthophosphoric acid. All these solutions and dilutions had to have the specific gravity of the urine, 1.020. This specific gravity was secured by using a 2.5 per cent sodium chloride solution, instead of water. Half-molar solutions of the phosphates and of the phosphoric acid standard solutions had been diluted with the sodium chloride solution in such a way that the smallest amount used was one one-hundredth of the standard solution. Increasing quantities were used according to tables 1 to 4. To 3 cc. of each dilution, 0.6 cc.

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3. Dowd, J. H.: New York M. Rec. **73**:768, 1908; **75**:763, 1909; **77**:1054, 1910; Illinois M. J. **56**:286, 1924.
4. Clemesha, cited by Dowd.[3]

of Dowd's reagent was added. This reagent contains equal parts of 10 per cent solutions of ammonium chloride, magnesium sulphate and ammonium hydroxide in 2 ounces (52 cc.). The forms of the crystals precipitated in the different solutions have been studied, after allowing at least ten minutes for their formation.

TABLE 1.—*Titration of Crystallized Trisodium Phosphate ($Na_3PO_4.12\ H_2O$)*

Cc. of M/2 Na_3PO_4	Gm. of Na_3PO_4	Na	P	O	Crystal Water	Type of Crystal
0.01	0.0019	0.000345	0.000155	0.00032	0.00108	6
0.02	0.0038	0.000690	0.000310	0.00064	0.00216	6
0.03	0.0057	0.001035	0.000465	0.00096	0.00324	5
0.04	0.0076	0.001380	0.000620	0.00128	0.00432	5
0.05	0.0095	0.001725	0.000775	0.00160	0.00540	5
0.06	0.0114	0.002070	0.000930	0.00192	0.00648	5
0.07	0.0133	0.002415	0.001085	0.00224	0.00756	5
0.08	0.0152	0.002760	0.001240	0.00256	0.00864	5
0.09	0.0171	0.003105	0.001395	0.00288	0.00972	5
0.10	0.0190	0.003450	0.001550	0.00320	0.01080	5
0.11	0.0209	0.003795	0.001705	0.00352	0.01188	4
0.12	0.0228	0.004140	0.001860	0.00384	0.01296	3
0.13	0.0247	0.004485	0.002015	0.00416	0.01404	3
0.14	0.0266	0.004830	0.002170	0.00448	0.01512	3
0.15	0.0285	0.005175	0.002325	0.00480	0.01620	3
0.16	0.0304	0.005520	0.002480	0.00512	0.01728	3
0.17	0.0323	0.005865	0.002635	0.00544	0.01836	2
0.18	0.0342	0.006210	0.002790	0.00576	0.01944	2
0.19	0.0361	0.006555	0.002945	0.00608	0.02052	2
0.20	0.0380	0.006900	**0.003100**	0.00640	0.02160	1

TABLE 2.—*Titration of Crystallized Disodium Hydrogen Phosphate ($Na_2HPO_4.12\ H_2O$)*

Cc. of M/2 Na HPO	Gm. of Na HPO	Na	H	P	O	Crystal Water	Type of Crystal
0.01	0.00179	0.00023	0.000005	0.000155	0.00032	0.00108	6
0.02	0.00358	0.00046	0.000010	0.000310	0.00064	0.00216	6
0.03	0.00537	0.00069	0.000015	0.000465	0.00096	0.00324	6
0.04	0.00716	0.00092	0.000020	0.000620	0.00128	0.00432	6
0.05	0.00895	0.00115	0.000025	0.000775	0.00160	0.00540	5
0.06	0.01074	0.00138	0.000030	0.000930	0.00192	0.00648	5
0.07	0.01253	0.00161	0.000035	0.001085	0.00224	0.00756	5
0.08	0.01432	0.00184	0.000040	0.001240	0.00256	0.00864	5
0.09	0.01511	0.00207	0.000045	0.001345	0.00288	0.00972	4
0.10	0.01790	0.00230	0.000050	0.001550	0.00320	0.01080	4
0.11	0.01969	0.00253	0.000055	0.001705	0.00352	0.01188	4
0.12	0.02148	0.00276	0.000060	0.001860	0.00384	0.01296	4
0.13	0.02327	0.00299	0.000065	0.002015	0.00416	0.01404	3
0.14	0.02506	0.00322	0.000070	0.002170	0.00448	0.01512	3
0.15	0.02685	0.00345	0.000075	0.002325	0.00480	0.01620	3
0.16	0.02864	0.00368	0.000080	0.002480	0.00512	0.01728	3
0.17	0.03043	0.00391	0.000085	0.002635	0.00544	0.01836	3
0.18	0.03222	0.00414	0.000090	0.002790	0.00576	0.01944	2
0.19	0.03401	0.00437	0.000095	0.002945	0.00608	0.02052	2
0.20	0.02580	0.00460	0.000100	**0.003100**	0.00640	0.02160	1

Eight different types of crystals can be differentiated (fig. 1). The dilutions of the phosphoric acid contain all eight types; the monosodium-phosphate dilutions contain only seven; the dilutions of the disodium phosphate and of the neutral phosphate, only six (tables 1, 2, 3 and 4). The differences are caused by gradually increased intervals of the change from one type to the next one, the smallest intervals being in the phosphoric acid solutions. As the acid gave the complete series of crystal

types, it is evident that the anion, and not the cation, is decisive for the form of the crystal type. The absolute quantities of each element (sodium, hydrogen, phosphorus, oxygen) in each of the dilutions can be learned from tables 1 to 4. It can easily be stated that the form of the crystal depends primarily on the amount of phosphate ($^{---}PO_4$)

TABLE 3.—*Titration of Crystallized Sodium Dihydrogen Phosphate* ($NaH_2PO_4.H_2O$)

Cc. of M/2	Gm. of			P	O	Crystal Water	Type of Crystal
0.01	0.00069	0.000115	0.00001	0.000155	0.00032	0.00009	7
0.02	0.00138	0.000230	0.00002	0.000310	0.00064	0.00018	6
0.03	0.00207	0.000245	0.00003	0.000465	0.00096	0.00027	6
0.04	0.00276	0.000360	0.00004	0.000620	0.00128	0.00036	6
0.05	0.00345	0.000475	0.00005	0.000775	0.00160	0.00045	6
0.06	0.00414	0.000590	0.00006	0.000930	0.00192	0.00054	6
0.07	0.00483	0.000705	0.00007	0.001085	0.00224	0.00063	5
0.08	0.00552	0.000920	0.00008	0.001250	0.00256	0.00072	5
0.09	0.00621	0.001035	0.00009	0.001395	0.00288	0.00081	5
0.10	0.00690	0.001150	0.00010	0.001550	0.00320	0.00090	5
0.11	0.00759	0.001265	0.00011	0.001705	0.00352	0.00099	4
0.12	0.00828	0.001380	0.00012	0.001860	0.00384	0.00108	4
0.13	0.00897	0.001495	0.00013	0.002015	0.00416	0.00117	4
0.14	0.00966	0.001610	0.00014	0.002170	0.00448	0.00126	3
0.15	0.01035	0.001725	0.00015	0.002325	0.00480	0.00135	3
0.16	0.01104	0.001840	0.00016	0.002480	0.00512	0.00144	3
0.17	0.01173	0.001955	0.00017	0.002635	0.00544	0.00153	3
0.18	0.01242	0.002070	0.00018	0.002770	0.00576	0.00162	2
0.19	0.01311	0.002185	0.00019	0.002945	0.00608	0.00171	2
0.20	0.01380	0.002300	0.00020	**0.003100**	0.00640	0.00180	1

TABLE 4.—*Titration of Phosphoric Acid*

Cc. of M/2 H PO	Gm. of H PO	H	P	O	Type of Crystal
0.01	0.00049	0.000015	0.000155	0.00032	8
0.02	0.00098	0.000030	**0.000310**	0.00064	7
0.03	0.00147	0.000045	0.000465	0.00096	7
0.04	0.00196	0.000060	**0.000620**	0.00128	6
0.05	0.00245	0.000075	0.000775	0.00160	6
0.06	0.00294	0.000090	0.000930	0.00192	6
0.07	0.00343	0.000105	**0.001085**	0.00224	5
0.08	0.00392	0.000120	0.001240	0.00256	5
0.09	0.00441	0.000135	**0.001395**	0.00288	4
0.10	0.00490	0.000150	0.001550	0.00320	4
0.11	0.00539	0.000165	0.001705	0.00352	4
0.12	0.00588	0.000180	0.001860	0.00384	4
0.13	0.00637	0.000195	**0.002015**	0.00416	3
0.14	0.00686	0.000210	0.002170	0.00448	3
0.15	0.00735	0.000225	0.002325	0.00480	3
0.16	0.00784	0.000240	0.002480	0.00512	3
0.17	0.00833	0.000255	0.002635	0.00544	3
0.18	0.00882	0.000270	**0.002790**	0.00576	2
0.19	0.00931	0.000285	0.002945	0.00608	2
0.20	0.00980	0.000300	**0.003100**	0.00640	1

present. A surprising statement could be made as to the appearance of the most rapidly formed crystal of type 1. This type forms in all the phosphates as well as in the phosphoric acid as soon as *the absolute quantity of phosphorus reaches 0.0031 Gm.* which figure, 31, is the atomic weight of phosphorus.

Dowd's reagent contains magnesium sulphate, ammonium chloride and ammonium hydroxide (a 10 per cent solution of each, equal amounts

in 2 ounces [59 cc.] [5]). If the absolute amount of ammonium magnesium sulphate that is present in 0.6 cc. of Dowd's reagent, the quantity used in the experiment mentioned, is calculated, *the figure 0.0358 is obtained, or 0.036, which is the molecular weight of ammonium magnesium sulphate (6 aqua).* It is therefore evident that the star crystal of type 1 forms if 1 atom of phosphorus meets 1 molecule of ammonium magnesium sulphate. These proportions are given in a solution in which the absolute quantity of phosphorus is 0.0031, and Dowd's reagent is added in the proportion of 1:5 (in my titration 0.6:3). A further increase of the amount of phosphates, or of phosphoric acid, did not change the type of the crystals, but they broke down into fragments and did not form when the amount of phosphates was too high.

THE CHARACTER OF THE CRYSTALS

The eight types of the crystals are of the same crystallographic system. They are isotropic. The crystal of type 1 is a feathered or skeleton crystal. It forms if there is a rapid deposition of the faces tending to build up toward a central point (Saylor [6]). The form of the crystal type 1 is caused by saturation or supersaturation, as crystals developing under metastable conditions tend to grow fastest at their angles, thus maintaining relatively few faces and a simple habit. If the rate of growth is too great, deposition of material at the edges and corners of the crystal exceeds that on its plane faces, and dendritic or skeletal habit will be produced.

The character of the crystals is the same at different specific gravities of the solution, but the form is incomplete. The crystals are fully developed at a specific gravity of 1.020. A few fragments of crystals are found at a specific gravity of 1.015, but the majority of the crystals are complete. About half the crystals are fragmented if the specific gravity is only 1.010; there are few complete crystals at a specific gravity of 1.005, and none are present at a specific gravity of 1.002.

A factor which may, however, influence the character of the crystals is the presence of larger amounts of dehydrating substances, i. e., substances which have a high solubility in water, such as urea and sugar. The changes in type caused by the presence of 0.3, 0.75, 1.4 and 2.8 Gm. of urea in a phosphate solution are drawn in figure 2 *A*. The crystals become smaller and poorer. These types may be found in urines of

5. Absolute quantities of ammonium magnesium sulphate in 0.6 cc. of Dowd's reagent:

Mg.	SO_4	$(NH_4)_2$	SO_4	$6H_2O$	Total
0.002	0.008	0.007	0.008	0.0108	0.0358

6. Saylor: J. Phys. Chem. **32**:1441, 1928.

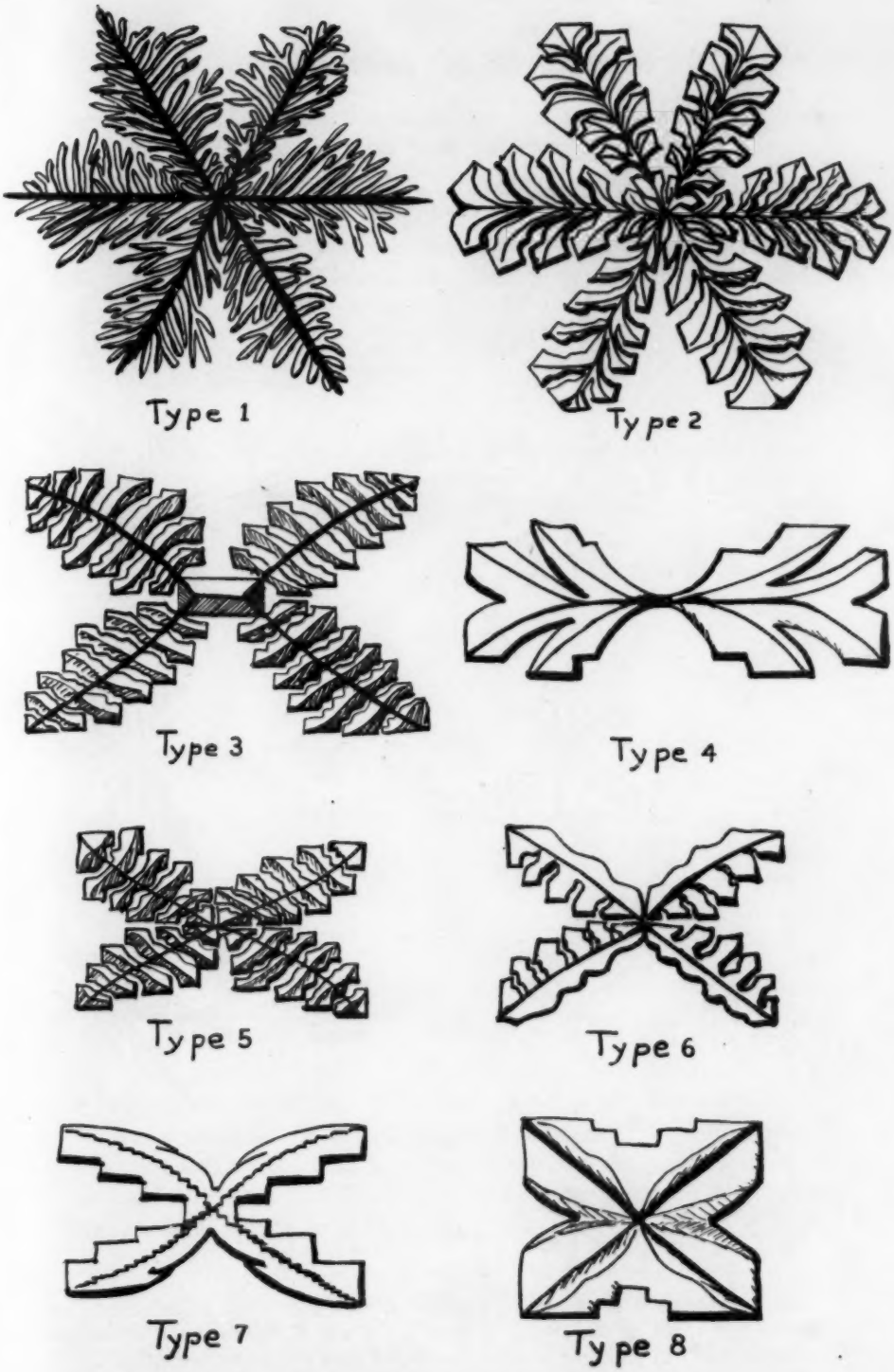

Fig. 1.—Eight types of ammonium magnesium phosphate crystals corresponding to the titration of phosphoric acid (table 4). Series of crystals: type 1, 15 by 15 mm.; type 2, 13 by 13 mm.; type 3, 7 by 10 mm.; type 4, 5 by 10 mm.; type 5, 5 by 10 mm.; type 6, 7 by 7 mm.; type 7, 5 by 7 mm., and type 8, 3 by 5 mm.

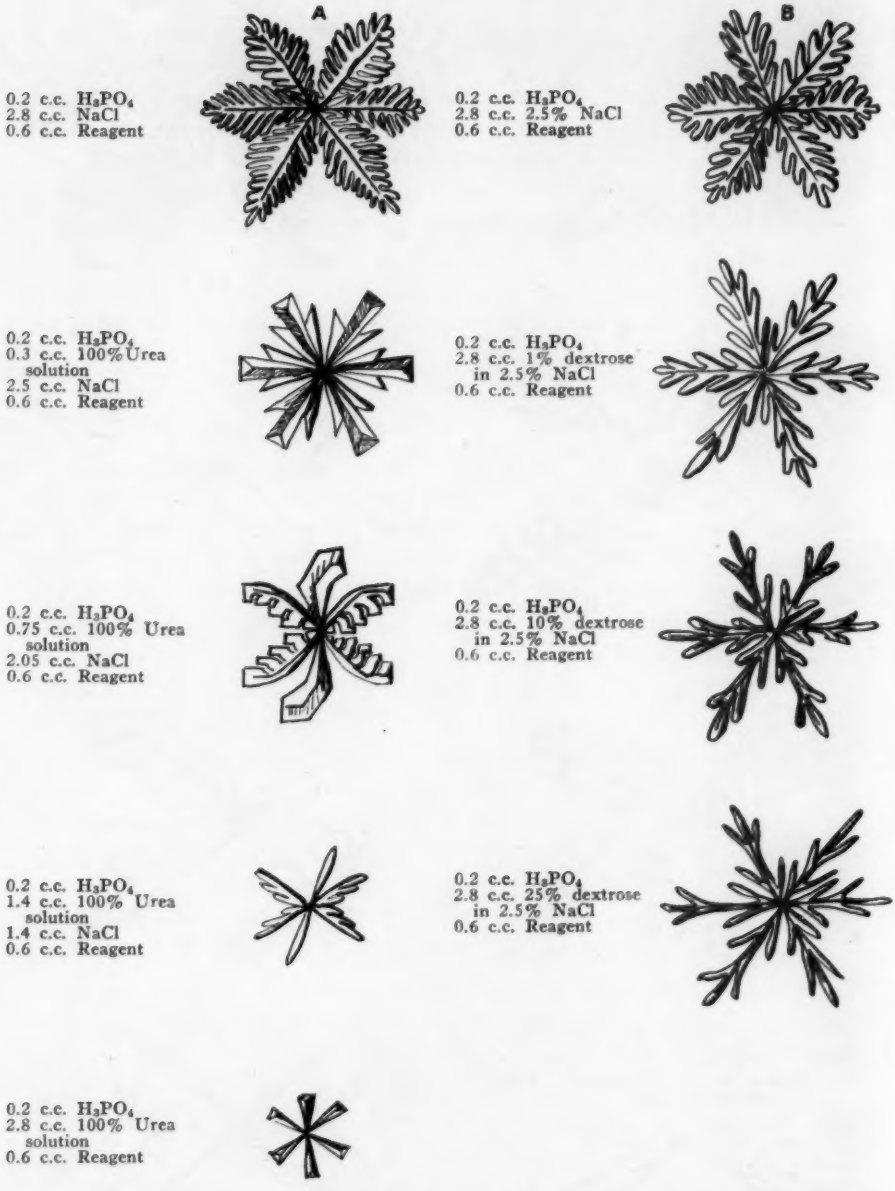

Fig. 2.—Influence of increasing amounts of urea (left) and sugar (right) on the size and shape of the crystal of type 1. *A* and *B* represent the unchanged crystal of type 1 as produced in solutions without urea and sugar, respectively.

uremic patients. Figure 2 *B* shows the changes in type in the presence of 1 per cent, 10 and 25 per cent dextrose. They may be found in diabetic urines.

THE PRACTICAL APPLICATION

Three quantitative methods may be used for the estimation of the phosphorus contents of the urine. The reagent (Dowd's reagent) is the same for all three methods.

Magnesium sulphate 10 per cent.....20 cc. Ammonium chloride 10 per cent.....20 cc. Ammonium hydroxide 10 per cent....20 cc.	or	Magnesium sulphate .....1 oz. Ammonium sulphate .....1 oz. Ammonium hydroxide 10 per cent..1 fluid oz. Distilled water.....8 fluid oz.

TABLE 5.—*Estimation of Phosphorus in Milligrams per Hundred Cubic Centimeters*

Types of Crystal	Mg. in 100 Cc.
1	More than 138
1 and 2	138-103
2 and 3	103- 93
3 and 4	93- 67
4 and 5	67- 46
5 and 6	46- 36
6 and 7	36- 23
7 and 8	23- 10
8	Less than 10

TABLE 6.—*Crystal Types with the Smallest Amounts of Phosphorus That Will Produce Them*

Crystal Type	Gm. of Phosphorus in 3 Cc.	Mg. in 100 Cc.
1	0.00310	103
2	0.00279	93
3	0.00201	67
4	0.00139	46
5	0.00108	36
6	0.00062	23
7	0.00031	10
8	Less than 0.00031	Less than 10

These reagents form a cloudy precipitate and should not be used before the precipitate has sedimented (after a few hours).

The reagent has to be well stoppered in order to prevent evaporation of ammonium. The room temperature at which the reaction takes place may not exceed 85 F. (29.5 C.), as the melting point of the crystals is low, and they lose their form if that temperature exceeds 90 F. (32 C).

Routine Method.—Pipet 3 cc. of the urine into a small test tube (Wassermann test tube) and add 0.6 cc. of the reagent. Mix and let stand for at least ten minutes until the crystals are precipitated. If there is a very small amount of phosphates in the urine, it may be necessary to wait longer—an hour, even more. Put 1 drop of the sediment on a clean, dry slide, cover with a cover slip and examine with the 4 mm. objective. The amount of phosphorus can be estimated from the type of the predominating crystal, as given in table 5 and in figure 1.

Drop Method.—For a quick estimation, and in cases in which large series of urines are to be examined, the following rapid method may be used: Place 5 drops of the urine on a slide (hanging drop slide preferable) with a capillary pipet, add 1 drop of the reagent with a pipet of the same caliber, mix well, wait until crystallization is complete (at least from five to ten minutes), place a small drop of the mixture on another slide, cover with a cover glass and examine with the 4 mm. objective. The amount of phosphorus is estimated from the type of crystals and table 5.

Exact Quantitative Estimation by Titration.—The reagents are (1) Dowd's reagent as described and (2) a 2.5 per cent sodium chloride solution. Measure 3, 2, 1.5, 1 and 0.5 cc. into five small test tubes. With the 2.5 per cent sodium chloride solution make all the tubes up to a volume of 3 cc. Add 0.6 cc. of Dowd's reagent to each tube, mix well, allow to stand until precipitation is complete (at least ten minutes) and examine the crystals formed in each tube with the 4 mm. objective. The tube which contains the smallest amount of urine, and which still has the same type of crystals as the first tube, is taken to contain the smallest amount of phosphorus that will form that type of crystal. The amount of phosphorus is calculated from this tube by means of table 6. Example: Tube 1 contains crystals of types 2 and 3; tube 4 is the last tube containing crystals of type 2. The amount of phosphorus in the urine is, according to table 6, 0.00279 Gm. of phosphorus in 3 cc., or 0.093 Gm. in 100 cc. of the urine. The method has been compared with the colorimetric determination of phosphorus (LaMotte's outfit) and gave satisfactory results.

If a single specimen of the urine is used instead of the twenty-four hour specimen, the concentration or dilution of the urine may be estimated by its specific gravity in the following way: A specific gravity of from 1.020 to 1.015 is to be considered as normal. A specific gravity of 1.012 corresponds to the dilution 1:2; a specific gravity of 1.008, to the dilution 1:4; a specific gravity between 1.005 and 1.006, to the dilution 1:8, and a specific gravity of 1.002, to the dilution 1:16. If a twenty-four hour specimen is available, the total amount of phosphorus will be calculated from the total amount of urine.

SUMMARY

In precipitating phosphate from solutions of the specific gravity of urine and varying in concentration from twentieth to two-hundredth molar, by using an ammonium magnesium sulphate reagent, eight different forms of crystals are found, changing gradually at certain amounts of phosphorus. The form of these crystals can be used for an estimation of the amount of phosphorus in the urine (*a*) by using the predominant type of crystals, and (*b*) by titrating the urine and using the crystal which predominates in the undiluted urine as an indicator in the highest dilution in which it appears. The specific gravity of the urine and the presence of considerable amounts of urea and of sugar change the form of the crystals in a typical way.

PURIFICATION OF POLIOMYELITIC VIRUS

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The fact that experiments on viruses are generally carried out in the presence of proteins and other substances necessarily contained in emulsions of infected tissues may partially explain the variability of results. Because of the protective or inhibitive manifestations of these substances, it is desirable to remove them in order to arrive at truer conceptions of the behavior of viruses.

The phenomenon of adsorption has long been employed as a practical means of selective separation and recovery of substances from heterogenous biologic mixtures. Andrejew [1] showed that antibodies could be absorbed from serum on kaolin and kieselguhr. Lewis and Andervont [2] reported that the viruses of vaccinia and fowlpox were adsorbed by powdered Berkefeld filters, kaolin and charcoal and the virus of Rous' sarcoma on aluminum hydroxide. Since Willstätter and his school have developed methods for the purification of enzymes by adsorption and elution, many investigators have applied this technic to viruses and bacteriophage.[3]

Rhoads [4] used poliomyelitic virus adsorbed in alumina gel for immunization of monkeys. He observed that the virus was adsorbed and inactivated in acid solution, but that when the p_H was raised to neutral or the basic side, the virus was actively present in the supernatant liquid. However, he did not apply these observations toward purposes of purification.

In our preliminary trials, kaolin, kieselguhr, magnesium and aluminum hydroxide were tested for a suitable adsorbent for poliomyelitic virus. Alumina gel, type "C," prepared according to the method outlined by Willstätter,[5] proved to be the most effective. Since aluminum

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† Dr. Brebner died on Nov. 9, 1932.

1. Andrejew, P.: Arb. a. d. k. Gsndhtsamte. **33**:84 and 337, 1909.

2. Lewis, M. R., and Andervont, H. B.: Am. J. Hyg. **7**:505, 1927.

3. Gildmeister, E., and Herzberg, K.: Centralbl. f. Bakt. (Abt. 1) **91**:228, 1924; Kligler, I. J., and Olitzki, L.: Brit. J. Exper. Path. **12**:172, 1931.

4. Rhoads, C. P.: J. Exper. Med. **29**:59, 1931.

5. Willstätter, E., and Kraut, H.: Ber. d. deutsch. chem. Gesellsch. **56**:149, 1923.

hydroxide is electropositive, this is in harmony with the results of cataphoretic experiments in which the virus was recovered at the anode.

At this point the work was suspended for other duties during the epidemic of poliomyelitis in New York in the summer of 1931. Anti-poliomyelitis horse serum [6] developed and used at this time was thought to contain antibodies against nerve tissue, stimulated, in the course of immunization, by the nerve tissue present in the inoculum (emulsions of infected monkey cord). This enhanced the necessity for a protein-free antigenic virus. With our permission, Sabin [7] completed and reported the successful adsorption and elution of poliomyelitic virus with aluminum hydroxide. We have since prepared quantities of purified virus largely for immunization of monkeys and horses.[8]

Since quantitative studies with viruses are obviously impossible at present, questions as to optimum concentration, time, temperature and p_H can be only roughly approached. From our numerous preparations we have in some instances reached close approximations to these important points.

Complete adsorption takes place when equal volumes of gel [9] and virus [10] are mixed. This is apparent from the fact that the supernatant liquid is not infective when a monkey is intracerebrally inoculated with it. When the quantity of virus greatly exceeds that of the gel, adsorption is incomplete. One hour is sufficient for adsorption and one for elution, but several hours are advisable to insure completion of reactions.

Because of the thermolability of the virus, the mixtures were kept in the cold room (50 F. [10 C.]) or refrigerator (4 C.), there being no perceptible difference whether the process was carried out in one or the other.

At p_H 7 adsorption is incomplete. The optimum range is apparently p_H 5.8 to 6.4, for within it the supernatant liquid is nearly always free from virus. As the p_H approaches 7 or goes toward 5, the supernatant become infectious. Contrary to the observations of Rhoads,[4] we have found the gel infectious when virus was adsorbed on it, both at p_H 5.4 and at p_H 6.2, so that adsorption in these instances did not seem to inactivate the virus. This inconsistency may be due, however, to variations in the physical characteristics of the aluminum hydroxide.

Elution takes place above p_H 7. In view of the large number of consistently successful elutions made within the p_H range of 8 to 9,

6. Weyer, E. R.; Park, W. H., and Banzhof: J. Exper. Med. **53**:553, 1931.

7. Sabin, A. B.: Proc. Soc. Exper. Biol. & Med. **29**:59, 1931; J. Exper. Med. **56**:307, 1932.

8. Brebner, W. B., and Schaeffer, M., to be published.

9. Standardized to contain about 22.5 Gm. of aluminum oxide per liter.

10. Berkefeld V filtrate of a 5 per cent emulsion of infected monkey cord in physiologic solution of sodium chloride.

this range can be said to be efficacious, though the virus can be eluted at higher p_H. One elution was carried out at p_H 10, giving a yield of virulent virus which retained its activity in that medium for several days. Though the bulk of the virus is probably released in the first elution, more virus can be recovered from the gel on subsequent addition of a dilute alkali solution, but the concentration of virus in the second "eluate" is greatly diminished.

METHOD OF PURIFICATION

Our usual procedure for the preparation of purified virus is as follows:

Pieces of spinal cord, taken from monkeys which succumbed to, or were killed at the height of, poliomyelitis, are triturated with sand in a mortar and suspended in physiologic solution of sodium chloride to make a 5 per cent suspension. If glycerolated cord is used, it is first washed with sterile water or saline solution to remove the glycerin. The suspension is well centrifugated and filtered through a Berkefeld V candle, and the filtrate is added to an equal amount of alumina gel [11] acidified with sodium or potassium acid phosphate to about p_H 6. The mixture is thoroughly agitated for several minutes and placed in the refrigerator. In the evening it is centrifugated, and the supernatant fluid is poured off and replaced with an equal volume of fifteenth-molar sodium hydrogen phosphate after the gel has been washed with distilled water. This brings the p_H to about 8.5. The mixture is again vigorously shaken and replaced in the refrigerator, where it remains over night. In the morning, the "eluate" is separated by centrifugation and is filtered through a Berkefeld N candle.

Such preparations give negative biuret, xanthoproteic and ninhydrin reactions and yet are highly infective. On two occasions, solutions concentrated by boiling in vacuo gave positive ninhydrin reactions, but the gel used in these preparations was several weeks old. Ninhydrin tests repeated with "eluates" from freshly prepared gel were negative. The work here needs further elaboration before conclusions can be drawn.

In efforts to extract the lipids from cord emulsions, as a possible intermediate step in purification, it was found that ether has no destructive effect on the virus even when extraction is prolonged over a period of two days. The emulsion is thoroughly shaken with ether in a separatory funnel. After several hours, separation takes place in three distinct layers, ether above, tissue débris in the center and a clear solution below containing the active virus. The clear solution is withdrawn and can be effectively adsorbed and eluted on alumina gel giving an excellent yield of potent virus.

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11. Aluminum hydroxide "C" easily suffers alteration. Consequently only fresh preparations or those not too old should be used.

Because purified preparations of enzymes are extremely unstable and are readily inactivated in a short period, it was thought that this might also apply to purified virus. Results thus far have been too variable to establish definite limits. In several instances, purified preparations became inactive after from eight to fourteen days. At the time of this writing, however, one preparation is still active after twenty-one days.

OTHER ATTEMPTED METHODS OF PURIFICATION

A long series of experiments was carried out at about the time that Clark [12] reported his experiments showing the virus to be associated with the "pseudoglobulin" fraction. Our approach was somewhat different, but the conclusions were similar. We took either a well centrifugated or a Berkefeld V filtrate of a 10 per cent emulsion of highly infective monkey cord and saturated it with ammonium sulphate. The precipitate came out rapidly, but from three to four hours were usually allowed for complete precipitation. The precipitate was then removed by centrifugation, placed with a few cubic centimeters of water in a dialyzing sac of goldbeater's skin and dialyzed against running tap water until free from ammonium sulphate. The volume within the sac increased during dialysis, and the water-soluble protein fraction again went into solution. When the contents of the sac were centrifugated and the clear supernatant fluid was Berkefeld-filtered, we had an extremely potent virus.

Attempts to precipitate the proteins from virus emulsions with basic lead acetate, ferric sulphate + barium carbonate, tungstic acid, etc., gave results wholly lacking in uniformity. Either such treatment is too drastic or the virus is firmly bound to the protein particles, with which it is carried out of solution. Our experiments with acetone on virus are not in accord with those of Howitt,[13] who reported successful precipitation of virus from filtrates with equal volumes of acetone.

No measure of success has been attained in efforts to apply Vinson's methods for purification of plant viruses [14] to the virus of poliomyelitis. Results obtained with safranin, however, though inconsistent in some respects, may bear significance and warrant reporting part of the protocols in detail (table). A recently modified technic of Vinson [15] was followed, except for changes in the proportions of safranin. It con-

12. Clark, P. F.; Schindler, J., and Roberts, D. J.: J. Bact. **20**:213, 1930.

13. Howitt, B.: Proc. Soc. Exper. Biol. & Med. **28**:158, 1930.

14. Vinson, C. G., and Petre, A. W.: Botan. Gaz. **87**:14, 1929; Logkin, M., and Vinson, C. G.: Contributions from Boyce Thompson Institute, 1931, vol. 3, p. 147.

15. Vinson, C. G.: Personal communication.

sisted chiefly of treating Berkefeld V filtrates of 5 per cent cord emulsions with 1 per cent aqueous solutions of safranin (Grübler). Increases in the amounts of safranin beyond 2 cc. to 10 cc. of filtrate did not visibly increase the precipitate. Since precipitation was extremely slow, it was allowed to take place over night in the cold room (50 F. [10 C.]). The mixtures were centrifugated, the supernatant fluid was poured off and the precipitate disintegrated, washed with distilled water several times and resuspended in physiologic solution of sodium chloride. Both fractions were then tested for the presence of virus. To remove the safranin from solution, a specially prepared fullers' earth (Lloyd's reagent) was used as adsorbent.

Experiments in Precipitation of Poliomyelitic Virus by Means of Safranin

Experiment	Preparation	Fraction Tested	Further Treatment	Amount Inoculated, Cc.	Monkey Inoculated	Result of Intracerebral Inoculation
1	10 cc. virus filtrate + 1 cc. safranin solution	Supernatant	None	1	263	Typical poliomyelitis (7 days)
		Supernatant	Cleared of safranin with Lloyd's reagent	1	260	No symptoms
		Precipitate	Washed and resuspended in physiologic solution of sodium chloride	1	264	Paralysis of hindlegs (10 days); recovery, with partial residual paralysis
		Precipitate	Resuspended and adsorbed and eluted on aluminum hydroxide	1	271	No symptoms
	Virus control					Typical poliomyelitis (11 days)
2	10 cc. virus filtrate + 2 cc. safranin*	Supernatant	None	1	283	Rise in temperature and tremors (4th to 6th day); quick return to normal
		Supernatant	Cleared with Lloyd's reagent	1	285	No symptoms of poliomyelitis; died of intercurrent infection (12th day)
		Supernatant	Adsorbed and eluted on aluminum hydroxide	1	286	No symptoms
		Precipitate	Washed and resuspended in physiologic solution of sodium chloride	1	284	Paralysis of right arm, left arm and leg; recovery with slight residual paralysis in left arm and leg
	Virus control					Typical poliomyelitis (6 days)
3	10 cc. virus filtrate + 4 cc.* safranin solution†	Supernatant	None	2	316	Partial paralysis of left side after 14 days; complete recovery
		Precipitate	Washed and resuspended in physiologic solution of sodium chloride	2	321	Typical poliomyelitis (8 days)
	Virus control					Typical poliomyelitis (7 days)

* More safranin added after contrifugation without further precipitation.

† Safranin solution in this experiment was about 1 month old. In experiments 1 and 2 it was freshly prepared.

Considering that the strain of virus used was extremely virulent, and fatal in nearly every case, it is especially significant that in these experiments, out of six monkeys infected with safranin preparations, four recovered; two with mild residual paralysis and two completely from unquestionable abortive attacks. Whether that is due to a direct virucidal or attenuating effect of the safranin, or a partial adsorption and resulting inactivation on the safranin, is not clear. However, since safranin, instilled by lumbar puncture into the spinal canals of three monkeys in the preparalytic stage, failed to arrest the action of the virus or in any way retard the progress of the disease, the latter explanation is perhaps the more probable. This is strengthened by the fact that the supernatant liquid, previously quite infectious, becomes inert when cleared of safranin with Lloyd's reagent, and that the virus cannot be released from the safranin by adsorption and elution on alumina gel.

SUMMARY

Poliomyelitic virus can be adsorbed and "eluted" on aluminum hydroxide, yielding a potent virus solution which gives no reactions for protein unless the solution is concentrated, when the results of the tests are sometimes positive. The optimum p_H for adsorption is apparently between 5.8 and 6.4; for elution, between 8 and 9.

Lipids can be extracted with ether from cord emulsions without injury to the virus. The extracted solutions can subsequently be effectively adsorbed and "eluted" on alumina gel.

Purified preparations are probably unstable, but the period of inactivation has not yet been definitely limited.

Attempts to purify the virus of poliomyelitis by several other methods have been unsuccessful. The results obtained with safranin may nevertheless prove interesting because the virus is definitely attenuated.

PULMONARY VARIX WITH SPONTANEOUS RUPTURE AND DEATH

REPORT OF A CASE

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Venous varices or circumscribed phlebectasias are commonly encountered and for the most part develop as the result of a congenital defect, a high venous pressure in a limited portion of the circulation or an inflammatory weakening of the wall of a vessel. Venous dilatations on the systemic side are frequent and do not warrant comment. However, the discovery of a large varix in a pulmonary vein giving rise to clinical symptoms, and resulting in death by rupture, is worthy of recording.

That such a condition is most unusual is evidenced by the fact that a search of the literature yielded only two cases. These presented many points of similarity with the case at hand, and hence will be briefly summarized here. Neither, however, showed spontaneous rupture, this being secondary to other disease.

As far as we can determine, the first observation was made by Hedinger,[1] who described a pulmonary varix in a middle-aged woman who had complained of cough for years with little expectoration. She was pregnant seven times, and during the fifth term influenza developed with pleuritis and hemoptysis. The hemorrhages ceased after delivery and did not reappear until the two following pregnancies, when small amounts of blood were coughed up. Subsequently abdominal pains radiating to the region of the gallbladder and a high grade of anemia with jaundice led to an operation that revealed a duodenal ulcer 1 cm. in diameter. The patient died two days after operation, with symptoms of severe anemia. An autopsy disclosed massive collapse of the left lung, with a tuberculous focus in the apex. In the anterior portion of the right lower lobe, and presenting on the surface, was a fluctuant saclike structure 4 cm. in diameter. This was covered by pleura and formed the outer wall of a blood-filled cavity partly sunken in the sub-

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1. Hedinger: Verhandl. d. deutsch. path. Gesellsch. **11**:303, 1907.

stance of the lung and communicating with a large pulmonary vein. The cavity was composed of smaller cavities from 2 to 0.7 cm. in diameter and had a smooth lining except for some deposits of fibrin. Infarcts were found in the spleen and in the kidneys. Microscopic examination confirmed the finding of tuberculosis in the upper left lobe. Sections of the wall of the varix showed fibrin deposits on the intima, muscle fibers, some elastic tissue fibrils and blood vessels, along with compression of the surrounding substance of the lung.

The second case was reported by Nauwerck.[2] A woman, 45 years of age and in perfect health, suffered a sudden attack of hemoptysis. A year later she experienced a second attack followed by four others, one of which was nearly fatal. After a period of good health she died, following eight days of small but continued hemorrhage. Autopsy revealed a thin-walled, white, nontransparent, bleblike structure, the size of a hazel-nut, on the lateral surface of the right lower lobe of the lung. Dissection showed it to be the outer wall of an oval cavity within the lung. A canal passed from the cavity to a branch of the right pulmonary vein, and the bleb and the cavity alike were lined by a delicate, smooth membrane, which became continuous with the intima of the vein into which it emptied. The cavity or varix was found to communicate with a branch of a bronchus of the first order, as the result of erosion.

REPORT OF CASE

Mrs. E. E., aged 46, white, of Hungarian descent, a farmer's wife, was first seen on April 17, 1930, by Dr. L. W. Gorham in consultation. She complained of recurrent attacks of difficult breathing and was admitted to the Albany Hospital for observation. Her illness dated back over a period of two years and began with a sense of suffocation and of oppression beneath the sternum, extending across the lower part of the left side of the chest and associated with dyspnea. Sitting up and bending forward or leaning over the back of a chair afforded slight relief. At first, the attacks occurred every ten or twelve days, but during the few months preceding admission were more frequent, and usually came on at night while she was in bed. On Jan. 21, 1930, she experienced a continuous dull ache in the left shoulder, extending down the left arm. This bore no relation to an attack of dyspnea. About February 1, ten days later, she had another attack of respiratory difficulty, following which the pain in her shoulder and arm subsided, never to return. Her family physician thought that the attacks were possibly due to asthma and on several occasions gave injections of epinephrine hydrochloride, which afforded no relief.

Her past history included the usual diseases of childhood. She had had four pregnancies and no miscarriages. In 1923, she had had pneumonia which was said to have been bilateral in the upper lobes.

Physical examination on April 17, 1930, showed a well developed, somewhat undernourished woman of middle age lying in bed. Her lips and finger-nails were

2. Nauwerck, C.: München. med. Wchnschr. **33**:1084, 1923.

cyanotic. There was neither dyspnea nor orthopnea. The pupils were equal and regular and reacted normally to light and accommodation. The thyroid gland was not enlarged, and there were no visible pulsations in the vessels of the neck.

The chest was normal. The heart was not enlarged to percussion, and there was no increase in mediastinal dulness. The cardiac sounds were normal, except for accentuation of the aortic and pulmonic second sounds. The blood pressure in the right arm was 210 systolic and 130 diastolic, and in the left arm, 200 systolic and 130 diastolic. The peripheral blood vessels were palpable, but not markedly thickened or tortuous. There was a hyperresonant note over the entire right side of the chest; the breath sounds were clear, and there were no râles. On the left there was dulness over the lower third anteriorly and posteriorly. Breath sounds, whispered voice and tactile fremitus were decreased over this area, and a few moist râles were noted. No egophony was elicited above the area of dulness. The abdomen was not remarkable.

A roentgenogram of the chest on April 19, 1930, showed evidence of a small effusion at the base of the left side of the chest. The cartilage of the first rib was heavily calcified on the left side, and posterior to it was a small, oval, opaque area just above the aortic arch, the significance of which was not clear.

About 600 cc. of bloody fluid was aspirated from the left side of the chest. Pathologic examination showed numerous mesothelial cells, many lymphocytes and polymorphonuclear leukocytes and no mitotic figures. Organisms were absent from smears. Cultures were negative, and inoculation of a guinea-pig revealed no evidence of tuberculosis.

A second roentgen examination on April 19, 1930, immediately following aspiration, showed a shadow measuring 4 by 5 cm., in the left infraclavicular region, lateral and anterior to the arch of the aorta.

The Wassermann reaction of the blood was negative. The hemoglobin content was 90 per cent. During the patient's stay in the hospital her temperature was normal, her pulse rate 80 and her respirations 20. The general opinion was that the patient had a malignant growth arising in the left lung. Treatment with radium or deep roentgen therapy was advised. She refused treatment, went home and was apparently free from symptoms for about one year.

The patient was not seen again until Oct. 5, 1931, when she returned for treatment. In May, 1931, her right leg became swollen, but later this swelling subsided. After August, she was confined to her bed because of dyspnea. Physical examination on Oct. 6, 1931, revealed a temperature of 99 F., a pulse rate of 90 and of poor quality, and a respiration rate of 20. The patient was seen propped up in bed, showing much emaciation. There was extremely marked purplish cyanosis of the lips, tongue and finger-tips, quite out of proportion to the degree of dyspnea. The pupils were normal and equal and reacted to light and in accommodation. The muscles of the neck were prominent, and there were no visible pulsations. There were deep supraclavicular and infraclavicular fossae. The heart was definitely enlarged. The apex was palpable, but not visible in the sixth interspace in the anterior axillary line. There was also increased dulness across the great vessels at the base. The cardiac sounds were normal, of regular rhythm and free from murmurs. There was no pulse deficit. The blood pressure was 210 systolic and 140 diastolic.

The left side of the chest was hyperresonant posteriorly as far down as the fifth dorsal vertebra, with dulness at the base. The breath sounds were harsh above and markedly diminished below. No râles were present. The abdomen was normal. The extremities showed slight pitting edema over both tibiae.

A roentgen examination on Oct. 6, 1931, revealed the shadow previously visible in the left side of the chest definitely increased in size. The heavy shadow of the mass visualized on April 19, 1930, had decreased in size, and confluent with it was a larger shadow of lessened density. Since the previous examination the heart had increased considerably in size. On April 19, 1930, it measured 16 cm. from base to apex, 11 cm. in its greatest transverse diameter and 8 cm. across the great vessels. At the time of the last examination it measured 19 cm. from base to apex, 14 cm. in its greatest transverse diameter and 9 cm. across the great vessels. There was symmetrical and general accentuation of lung markings, suggesting engorgement of the pulmonary circulation (fig. 1).

The patient had a comfortable first day in the hospital and slept quietly the first part of the night without medication. While being helped onto a bed-pan at

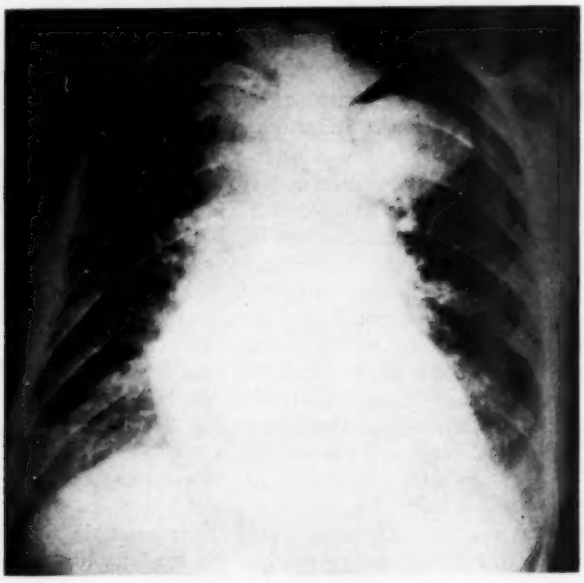

Fig. 1.—A roentgenogram taken in October, 1931. The "tumor mass" visualized seventeen months previously shows definite increase in size and measures 5.5 by 5 cm. The heart is much larger than at the previous examination.

3 a. m. she began to gasp for breath, put her hand over her left side as though in pain, and was unable to speak. The pulse became feeble; she gasped for breath several times and expired.

Findings at Autopsy.—The body measured 52½ inches (133.37 cm.) in length, was well developed and moderately obese. The legs and thighs were edematous. The skin was dark in color, suggestive of southern European parentage. The lips and finger-tips were cyanotic, and there was slight clubbing of the fingers.

The peritoneal cavity was free from fluid, and the lymph nodes showed no changes. The diaphragm reached the level of the fourth rib on the right, and on the left bulged downward into the peritoneal cavity to such an extent that its lowest point lay below the level of the ribs in the midclavicular line.

When the ribs on the left were cut through for removal of the breast-plate, large quantities of blood welled up through the incision. It came out with such

force as to indicate its being under considerable pressure. On removal of the breast-plate, the left pleural cavity was found completely filled with clotted and unclotted blood. A large amount of this was unavoidably lost, but 2,800 cc. was actually measured. It is estimated that about 500 cc. escaped. The left lung was partly collapsed because of the pressure of the large amount of blood. There were

Fig. 2.—A photograph of the left lung and the heart (slightly retouched). The varix is shown and a pulmonary vein (*P.V.*) leading into the left auricle (*L.A.*). (The arrow indicates the point of rupture of the varix.) The wall of the left ventricle is hypertrophied.

no adhesions. The right cavity was completely filled by its lung and was free from fluid and adhesions.

In the mediastinum, a small bit of thymic tissue was found embedded in fat, but otherwise it was not remarkable.

The pericardial cavity contained 75 cc. of clear, yellow fluid, and its lining was smooth and glistening, there being no adhesions or thickening of the pericardium.

The heart was removed along with both lungs and hence not weighed, but its weight was estimated at 400 Gm. It was very hard and had apparently stopped in systole. The musculature of the left ventricle averaged 17 mm. in thickness, and that of the right, 9 mm. The coronaries were free from change. The valve leaflets were negative.

The left lung was partially collapsed because of the large amount of blood in the left pleural cavity. On the medial surface of the upper lobe, about midway between the apex and the hilus, there was found a collapsed cystlike structure, which, when distended, measured 5 cm. in diameter and projected 4 cm. above the level of the pulmonary surface (fig. 2). Examination revealed a small, ragged

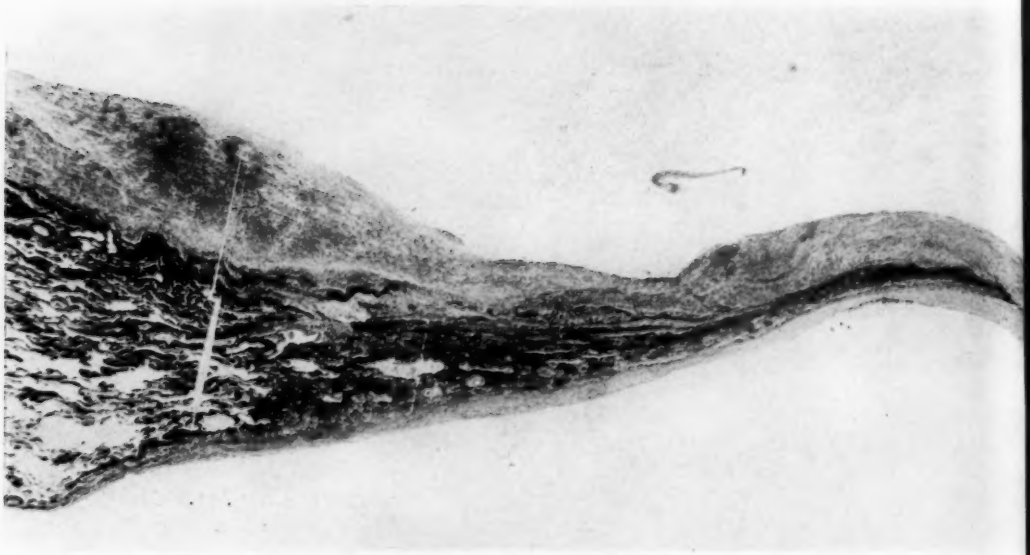

Fig. 3.—The thin wall of the varix at the right merges with the lung, and is completely covered with pleura. The elastic fibers continue out into the wall of the varix. A large organized mural thrombus is present; × 18.

tear in its upper surface, 5 mm. in length. When the opening was manipulated, blood flowed from it. The walls of this cystlike structure were less than 1 cm. in thickness and almost transparent. Small vessels could be seen coursing over its surface. The sac was opened by enlarging the tear already present, and there was brought to view a cavity in the substance of the lung, 4 cm. in diameter and 3 cm. in depth. This cavity, as well as the cystlike bleb, was lined by a smooth, white tissue, resembling the lining of a vein. The portion of the cavity within the substance of the lung was more or less imperfectly divided by numerous anastomosing trabeculae. Close examination revealed small deposits of fibrin. A blood vessel 1 cm. in diameter was found communicating with the cavity. On being traced downward, this vessel became larger in diameter and was found to be a pulmonary vein, which, after receiving numerous tributaries, emptied into the left atrium of the heart. No communication with a bronchus could be demonstrated.

Fig. 4.—The pulmonary varix in Hedinger's case, opened to show the character of the lining. It is located in the lower lobe of the right lung.

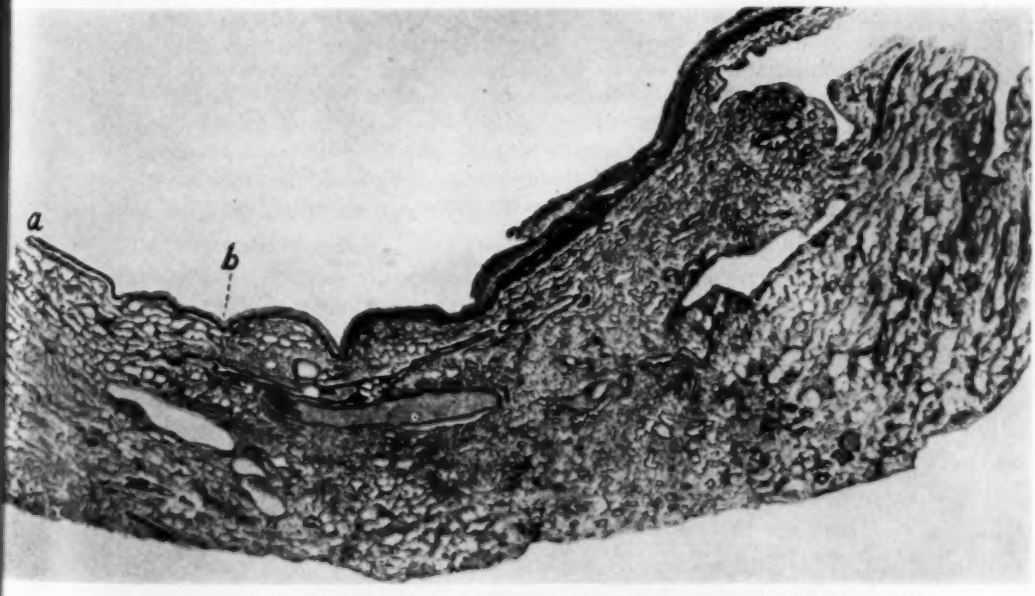

Fig. 5.—A section of the pulmonary varix in Hedinger's case; Weigert's elastic tissue stain; slightly magnified: (*a*) the pulmonary vein; (*b*) the beginning of the varix. A lamellated thrombus covers a portion of the lining at the right.

Further section of this lung showed a diminished amount of air and a pale, finely granular, very moist parenchyma.

The right lung weighed 500 Gm. It was pale and heavy and lacked crepitation in its posterior portions. On section, the parenchyma was finely granular and exuded a small amount of blood. The bronchial mucosa was everywhere congested, and the large vessels were free from anomalies.

The remaining findings were essentially negative.

Histologic Examination.—Sections were made from the wall of the varix itself, and from various levels of the large vein that drained the varix, as far down as its entrance into the left atrium. These were all stained with hematoxylin and eosin and with Verhoeff's stain for elastic tissue. Sections of the atrial wall were normal. Throughout the various sections of the vein were all the components of a normal pulmonary vein, except for a very thin layer of organized thrombus on the intima of a portion of the vein near the varix (fig. 3). A section showing the transition between the vein and the varix revealed in one place an extremely thinned-out wall over which was present a thick layer of organized thrombus. The thrombus was apparently thickest over the thinnest portion of the vein. Compressed parenchyma of the lung gave support to the wall of the vessel in its thinner portions. In this portion could be seen the junction of the pleura and the wall of the varix. A wedge-shaped bit of compressed and fibrotic lung lay between the uniting vascular wall and pleura. Elastic fibers could be traced from the wall of the vein proper into that of the varix, and could be demonstrated in all sections of the wall of the varix. The line of demarcation between the pleura and the wall of the varix could be clearly traced where these two structures united to form the cystlike dilatation. A great majority of the pleural mesothelial cells were unavoidably brushed off during preparation, but enough of them remained to prove that the varix was covered by a pleura apparently complete in all respects. Thus it is seen that the wall of the varix consisted of two united layers of tissue—the venous structures and the pleura. The venous portion of the wall of the varix varied considerably in thickness in different portions. In all sections of the wall studied, the pleural layer and the venous layer could be distinctly made out.

The pulmonary substance accompanying the sections studied showed slight evidence of old hemorrhage, but nothing indicative of old or recent inflammation. All sections revealed slight arterial thickening and definite phlebosclerosis. In one section (fig. 5) there were several vessels containing organized thrombi.

COMMENT

The gross and histologic studies indicate clearly that the bleblike structure was truly a local venous dilatation and not a cyst or other pathologic formation which had become united with the pulmonary vein. The presence of all of the coats of a vein, arranged in regular manner, was sufficient to establish this fact. Granted that it was a true venous varix, we must consider its etiology by comparing the etiologies of the more common varices of the venous systemic system.

There are three main theories as to the formation of these:

1. We find anomalous venous anastomoses in which one limb is subjected to increased venous pressure as the result of pressure from nearby anatomic structures, or as the result of an exceptionally narrow

orifice at the point where the anomalous limb joins a main trunk, or as the result of differences in pressure in the two subdivisions of the venous system united by such an anastomosis. Stasis results in such a limb, and a gradual local dilatation occurs. In some cases of this sort where very little venous flow is occurring, the portion of vein supplying the varix becomes atrophied and remains as a cordlike structure (Hasebroek [3]). The presence of a pulmonary varix on the surface of the lung could not conceivably have arisen under such circumstances.

2. Other systemic venous varices have been said to occur (in the lower extremity, particularly) because of insufficiency of the valves of the veins, giving rise to increased intravenous pressure. The pulmonary varix cannot be explained on the grounds of increased hydrostatic pressure, regardless of the presence or of the absence of venous valves, since its location represents the highest point of the pulmonary circulation. In the cases of Hedinger and Nauwerck, the varices were located in the lower lobe of the lung, but even so, it cannot be thought that increased hydrostatic pressure caused them.

3. The remaining theory of the development of venous varices has to do with inherent congenital or inherited weakness of the vascular wall. Circumscribed portions of a given vein may be lacking in certain elements, and the want of these will permit that portion of the vein to dilate under the pressure within it.[4] We cannot rule out such a factor in the formation of the varix in the present case, although its form does not lead one to believe that this is true. Kaufmann [5] maintained that inflammation near a vein may be communicated to it, causing a tendency to ectasia. No evidence of inflammation, either acute or chronic, could be found in the case under discussion.

Venous anomalies of the lung are rare, but there is one that is described several times in the literature. It is an anomalous pulmonary vein draining the left upper lobe of the lung and emptying into the systemic venous circulation (Jack and Lee [6]). Such an anomaly is readily explained on embryologic grounds, a discussion of which is beyond the scope of this report. Such an explanation does not seem applicable to the present case.

Since it is impossible to explain the formation of the varix on any of the grounds mentioned, a purely congenital anomaly must be considered. It will be noted that the pulmonary vein with which the varix communicates is the main venous channel of the left upper lobe. At the

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3. Hasebroek, K.: Frankfurt. Ztschr. f. Path. **22**:102, 1919.

4. Forst, A. W.: Frankfurt. Ztschr. f. Path. **17**:137, 1915. Bennett, W. H.: Congenital Sacculations and Cystic Dilatations of Veins, Lancet **1**:788, 1890.

5. Kaufmann, E.: Textbook of Pathology, translated by Reimann, Philadelphia, P. Blakiston's Son & Co., 1929, p. 161.

6. Jack, R., and Lee, H.: Anat. Rec. **39**:359, 1928.

point where the vein and the varix unite, the vein is fully 1 cm. in diameter. The varix is located on the surface of the lung; hence we face the unusual condition of a vein with a diameter of 1 cm., at the surface of the lung. Ordinarily, the main trunk of the vein of the left upper lobe is less than 1 cm. in diameter at about the midpoint of the lobe, and usually it has so many subdivisions at this location that it is difficult to follow. In this case, however, we find a huge, thick-walled vein traversing the entire left upper lobe and emerging on the surface into a large saccular varix. This vein received tributaries from the pulmonary substance all along its course. No veins were found emptying into the varix itself; hence the varix was filled, not by blood passing through it as in most varices, but merely by back flow from the large vein communicating with it. It cannot be said that the saccular varix was not the remnant of a fetal structure, but it is difficult to imagine what it could have been in the very early life of the patient.

Between the time of the first roentgen examination in April, 1930, and the second, seventeen months later, the shadow which proved to be the varix had definitely increased in size (fig. 1). Thus it is apparent that the bleblike structure was undergoing progressive increase in size. This observation raises the question whether or not the varix had been gradually increasing in size throughout the life of the patient. Such is, at times, the history of varices, and it may be presumed that in early childhood the varix had been quite small. The lack of symptoms until middle age tends to support the idea of continuous growth, for had the structure always been of such large size, it seems probable that it would have caused symptoms at an earlier date. The walls of the varix must be presumed to have suffered the same phlebosclerosis noted in the pulmonary veins elsewhere. With such sclerotic changes going on and the walls of the varix becoming less and less elastic and at the same time thinner, it is not surprising that a rupture occurred with the ever increasing blood pressure of advanced years. It will be recalled that the blood pressure was 210 systolic and 140 diastolic. Such pressures were naturally not present in the pulmonary circulation, but there must have been a certain proportional increase.

It is interesting to note that death occurred as a result of rupture of the varix into the pleural cavity. In the two cases previously cited, death resulted indirectly from the varix. Hedinger postulated that the duodenal ulcer in his case came as the result of an embolus arising in the varix and lodging in a duodenal vessel. The patient died following an operation for the symptoms caused by the ulcer. In Nauwerck's case, the patient died from a hemorrhage brought about through erosion of the wall of the varix by adjacent tuberculosis. Were it not for embolism in the first case and tuberculosis in the second, these patients might presumably have lived many years. The present case is unique

in that death resulted from an accident in the varix itself and not from other factors resulting from or acting on the varix.

Hedinger attributed the hemoptysis in his case to extravasation of blood from the varix into the surrounding substance of the lung and its subsequent expectoration. In Nauwerck's case, the preceding hemoptysis was clearly due to the tuberculous process which later eroded into the varix and caused fatal hemorrhage. In our case, no hemoptysis occurred.

It is not easy to explain the attacks of dyspnea by the pathologic findings. Roentgenograms show that the mesial margin of the varix was some distance from the trachea, and so compression of the latter by the varix was almost impossible. There is a question whether pressure on the vagus or phrenic nerves could have given rise to the respiratory distress. The vagus is mainly composed of parasympathetic fibers, and stimulation of these would cause spasm of the bronchial musculature, giving rise to dyspnea. The paroxysmal attacks of dyspnea in the beginning and continued dyspnea during the latter part of the illness suggest some effect on the nerve trunks by the gradually enlarging varix. The presence of a pleural effusion at the time of the first admission to the hospital, and its failure to recur after its removal is without explanation.

SUMMARY

A case of pulmonary varix in a woman 46 years of age is presented, with the findings at autopsy. Spontaneous rupture of the varix with massive hemothorax was the cause of death. There is evidence that the varix gradually increased in size, and that the resulting enlargement gave rise to symptoms. The rupture is believed to have occurred following progressive thinning of the wall of the varix during a prolonged period of hypertension. The various theories of the development of venous varices are discussed. A congenital anomaly of the left pulmonary vein seems the most probable explanation of this rare condition. This case is apparently the first to be recorded in the English literature.

RETENTION OF CONGO RED IN AMYLOID DISEASE

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Amyloid disease is commonly thought of as a general metabolic disturbance bringing about the accumulation of a peculiar firm, homogeneous, waxy and semitranslucent material in the organs. It was well described in the middle of the last century by Rokitansky and Virchow, the latter being responsible for its name. Rokitansky [1] in 1842 and Wagner [2] in 1861 described the renal involvement.

Because of its association with long-standing and wasting diseases—for example, tuberculosis, syphilis, leukemia, Hodgkin's disease, chronic ulcers and multiple myeloma—it is most often found in institutions for the care of chronic invalids. It is less common in recent years, owing to the improvement in the treatment of tuberculosis and to the more recent surgical methods in the treatment of chronic suppurative processes. The clinical diagnosis is suggested in the diseases mentioned, especially if a large liver or spleen is present. Calcification in the liver is often demonstrated by roentgenologic examination. Hemorrhage from the large bowel may occur late in the disease.

Persistent edema and albuminuria in the presence of a chronic disease point strongly to amyloid deposit in the kidneys. Casts are not common, and the appearance of granular casts is but a tardy indication of approaching uremia.[3] The blood pressure is not elevated. Larsen [4] reported a case in which the heart weighed 700 Gm., the result of local amyloid infiltration of the myocardium. The kidneys in this case were normal. The lipoids in the blood vary considerably, and all ranges have been reported. Labbé and his associates [5] observed a case of so-called lipoid nephrosis in which there were characteristics of amyloidosis, and he suggested a relationship between the two diseases.

From the Department of Pathology, University of Minnesota.

1. von Rokitansky, Carl: Handbuch der pathologischen Anatomie, Vienna, Braumüller & Seidel, 1842, vol. 3, p. 421.

2. Wagner, E., quoted by Noble and Major.[7]

3. Abrami, P.; Betrand-Fontaine; Lichtwitz, A., and Laudat, M.: Bull. et mém. Soc. méd. d. hôp. de Paris **47**:753, 1931.

4. Larsen, R. M.: Am. J. Path. **6**:147, 1930.

5. Labbé, Marcel; Boulin, R.; Azerad, and Justin-Besançon, L.: Bull. et mém. Soc. méd. d. hôp. de Paris **45**:1288, 1929. Labbé, Marcel; Boulin, R.; Justin-Besançon, L., and Zamfir: ibid. **47**:1092, 1931.

Bell [6] considered this type of renal disease an atypical nephrosis because of the absence of inflammatory changes within the glomeruli. The older conception of MacCallum that the renal insufficiency depends on an associated lesion of the kidney no longer holds. Noble and Major [7] found it to be primarily a vascular process involving the glomeruli, and they established it as a progressive lesion leading to renal insufficiency. A rise in urea nitrogen, a decrease in the output of phenolsulphonphthalein and the appearance of granular casts precede the uremic state.

A specific test was described by Bennhold [8] by which the diagnosis of amyloid disease is easily established. In principle, it depends on the adsorptive affinity of amyloid tissue for an intravital stain. The organic dye used is the sodium salt of diphenyl-diazo-binaphthionic acid, commonly known as congo red. The technic is simple, and the sources of error are few. They have been considered by Bennhold,[8] Labbé and his associates [5] and Barker and Snell.[9]

Amyloid has been found in every organ of the body. The most common sites are the spleen, kidneys, endocardium, liver and intestinal mucosa. Lymph nodes in the region of a tumor or a suppurative process often contain this material, especially if the lesion is situated in or about cartilage, for example, the larynx and bronchi. Paige [10] described a case of multiple myeloma with amyloidosis in which the Bence-Jones protein took the characteristic amyloid stains.

The material was long considered a compound protein combined with chondroitin sulphuric acid ($C_{18}H_{27}NSO_{17}$).[11] This acid is found normally in the aorta, elastic tissue and senile cartilages, but not in the parenchymatous organs. It is present in the blood in small amounts and is excreted by the kidney. In all forms of nephrosis there is an increase of the acid in the circulatory fluid. Hansen, in 1908, analyzed many amyloid organs in which this acid was not found. Clinical analysis of various organs of many patients reveals great variance in the elementary composition of amyloid, but seldom is carbohydrate present. It is a colloid protein, for the amyloid substrate gives the Abderhalden reaction when it is added to the blood serum of patients with chronic suppurative processes. There is no evidence that it is a nucleoprotein.

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6. Bell, E. T.: A Text-Book of Pathology, Philadelphia, Lea & Febiger, 1930, p. 315.

7. Noble, J. F., and Major, S. G.: Arch. Path. **8**:762, 1929.

8. Bennhold, Hermann: Deutsches Arch. f. klin. Med. **142**:32, 1923; Klin. Wchnschr. **3**:1711, 1924.

9. Barker, N. W., and Snell, A. M.: J. Lab. & Clin. Med. **16**:262, 1930.

10. Paige, B. H.: Am. J. Path. **7**:691, 1931.

11. Wells, H. G.: Chemical Pathology, ed. 2, Philadelphia, W. B. Saunders Company, 1914, p. 378.

The attraction of the substance for stains varies as does its composition. Iodine staining of the gross specimen depends on a physical rather than a chemical action, adsorption by amyloid being greater than that of the surrounding tissue. Congo red stains the tissues on section as well as intravitally.

Intravenous injections of foreign proteins (egg albumin, blood serum, nutrose) or of bacteria (Bacillus coli and staphylococci) result in the rapid formation of amyloid in the mouse. Chronic suppurative processes in the rabbit will produce the same result, unless the spleen has been previously removed. Splenectomy, however, does not alter the ability of the mouse to produce the substance. This rules out a responsible splenic ferment in the mouse.

Jaffé [12] stated his belief that the formation of amyloid is the result of hypersensitiveness to the injected substance. He produced amyloidosis in mice by muscular injection of nutrose for a certain period of time, and found that the reaction to protein changed during the course of the experiments. At first, there was a rise in temperature after each injection; later, from the fifth to the tenth week, the temperature fell after this procedure. It was during this later period that the animals began to lose weight, and amyloid soon developed.

The formation of amyloid can be delayed in the experimental animal in two ways: Jaffé [13] found that it was delayed by a diet rich in fat. Smetana [14] found that blocking the reticulo-endothelial system of the mouse with india ink would slow the production of this material.

REPORT OF A CASE

A woman, aged 47, had had chronic nonsuppurative polyarthritis for four years. She had been totally incapacitated by the deforming condition for one year. A test of renal function had not been made early in the course of the disease. Four months before her death, edema developed, which began in the lower extremities and progressed rapidly to anasarca in two weeks.

The blood pressure at this time (Aug. 4, 1931) was 120 systolic and 70 diastolic in millimeters of mercury, and there was a soft systolic blow over the apex of the heart. Many fine râles were heard over the basilar pulmonary areas. There was moderate pitting edema of the entire body. The urine contained abundant albumin on all occasions (varying from 2.9 to 3.86 Gm. in twenty-four hour specimens), but no casts or erythrocytes; from 20 to 30 leukocytes were found in a catheterized specimen. By dilution-concentration test the specific gravity varied between 1.009 and 1.015. The return of phenolsulphonphthalein in the urine was 22 per cent in two hours. The urea nitrogen was 9.45 per cent (9 mg.), the xanthoproteic nitrogen 18 units (normal), and the cholesterol 175 mg. in each 100 cc. of blood. The concentration of hemoglobin (Dare) was 67 per cent; erythrocytes numbered 4,000,000 and leukocytes 7,200 in each cubic millimeter of blood. The percentages

12. Jaffé, R. H.: Arch. Path. **1**:25, 1926.
13. Jaffé, R. H.: Arch. Path. **2**:149, 1926.
14. Smetana, H.: Proc. Soc. Exper. Biol. & Med. **24**:187, 1926.

of the various white cells were as follows: polymorphonuclear leukocytes, 58; lymphocytes, 40, and mast cells, 2. The basal metabolic rate was —25 per cent. The Wassermann reaction of the blood and that of the spinal fluid were negative. Two congo red tests were performed, one, one hundred and five days before death, and the other, eighty-four days before death. Fifty-six per cent of the dye disappeared from the blood stream in one hour on both occasions, and none was found in the urine.

The course of the disease was one of progressive weakness, persistent albuminuria and edema without elevation of the blood pressure, temperature, pulse rate or respiratory rate. Further laboratory observations were not made. The patient died on Nov. 25, 1931.

The body was extremely emaciated, weighing approximately 90 pounds (40.8 Kg.) and measuring 170 cm. in length. There was moderate generalized edema of the body, most noticeable in the lower extremities. The skin was waxy. The peritoneal cavity contained 1,000 cc. of clear watery fluid. The pleural cavities were partially obliterated by scattered firm, fibrous adhesions. The pericardial sac contained many adhesions of the same type. The heart weighed 200 Gm.; it did not show evidence of amyloid degeneration. The right lung weighed 300 Gm.; the left, 320 Gm. There was no evidence of tuberculosis or of an acute pulmonary process. The deposit of amyloid was greatest in the spleen and suprarenal glands, less in the kidneys, and least in the liver.

The following stains were used in the study of the spleen, kidney, suprarenal glands and liver: unstained intravital congo red, diluted hydrochloric acid added to unstained intravital congo red, congo red, hematoxylin alone, picric acid, methyl violet, sudan III, and azocarmine (Heidenhain's modification of Mallory's aniline blue as used by Bell [6] and McGregor [15]). The last stain proved to be of most value in microscopic study. Structural detail was excellently preserved, and the amyloid stained distinctly.

The spleen weighed 200 Gm. (fig.). It was very firm and cut with increased resistance. The capsule was smooth and tense, but not thickened. The cut surface was a translucent bright orange, and the corpuscles showed prominently as small bright red areas. Compound solution of iodine turned these brown. The addition of dilute hydrochloric acid changed the red areas to a dark blue, thus confirming the suspicion that the coloring of the corpuscles was due to the intravital staining of the congo red dye.

In the unstained frozen section the amyloid was red, but was unsatisfactory for microscopic study. The amyloid was distributed throughout the splenic tissue, but was most prominent in the malpighian corpuscles. It seemed more compact in the center of the corpuscles, and the central arterioles were almost obliterated, owing to subintimal deposits of amyloid. Occasionally a corpuscle was seen in which the first deposit of amyloid appeared to be placed peripherally, and a few revealed both processes. Fat stains showed the outer margins of the corpuscles to contain much fat, owing to the degeneration of compressed tissue pulp.

The liver weighed 700 Gm. It was small, pale and firm, and of a semitranslucent, soft brown color. The markings on the cut section were indistinct. The capsule was tense, but not thickened. There was no red tissue such as was found in the spleen, suprarenal glands and kidneys. However, congo red stained the amyloid tissue satisfactorily for microscopic study. Compound solution of iodine did not change the color of the surface, nor did iodine and sulphuric acid.

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15. McGregor, Leone: Am. J. Path. **5**:545, 1929.

Small deposits of amyloid were found scattered throughout. These were irregular in shape and placement. Confluent areas of amyloid were found in all portions of the lobules, and where they were most abundant, compression of the hepatic cords was observed to be causing atrophy and necrosis of hepatic cells. It seemed to be placed on the outer side of the endothelium of the sinusoids just beneath the hepatic cells. Little fat was present in any portion of the liver. No evidence of amyloid material was found in the cells of the reticulum. Occasionally crystal-like spicules of amyloid were noted, as described by Jaffé.

The kidneys each weighed 110 Gm. They were firm, pale and waxy. The capsules stripped easily, leaving pale, finely granular surfaces. The organs cut with greatly increased resistance, and each disclosed a thick pallid cortex containing small red spots throughout. Application of iodine to these areas brought out small

A section of the spleen, showing the congo red deposit (dark portions) in the amyloid tissue. Addition of hydrochloric acid turned the dark portions blue.

brownish-black spots grossly appearing to be the glomeruli. Addition of dilute hydrochloric acid to the fresh section changed the red spots to blue.

The amyloid deposit was easily seen in the glomeruli where it lay inside the basement membrane between the capillary endotheluim and the covering epithelium. In some areas it encroached on the capillaries to the point of closure. As atrophy of the glomeruli occurred, the space was filled with collagenous fibers staining blue with Mallory's aniline blue stain. Under hematoxylin and eosin, these fibers had the appearance of hyalin. They took none of the specific amyloid stains. All glomeruli were involved to greater or lesser degree.

The suprarenal glands each weighed 10 Gm. They were extremely firm. The cut section showed the surfaces to be a bright orange due to the intravital congo red stain. This was confirmed by the addition of dilute hydrochloric acid, which changed the color to blue. Six months in formaldehyde preservative did not change this reaction.

Microscopic examination showed most of the amyloid to be deposited in the zona fasciculata of the cortex beneath the endothelium of the capillaries.

The pancreas, stomach and small and large intestines did not contain amyloid deposit, nor did they manifest other pathologic change.

The urinary bladder contained a small amount of cloudy urine.

A small, benign cervical polyp was found. The tubes and ovaries revealed no abnormalities.

A few atheromatous plaques were present in the lower abdominal aorta.

Enlarged lymph nodes were not found.

The cavities of the joints did not contain suppurative processes.

The organs of the head and neck were not examined.

COMMENT

It is possible that this patient died in uremia, although the terminal changes in the chemical elements of the blood were not studied. The liver contained less amyloid and less of the dye than did any of the other organs. Jaffé [13] found that the endothelial cells of the liver were active in removing amyloid from that organ. Likewise, the liver removes congo red from the blood serum and excretes it in the bile.

Bookman and Rosenthal [16] at necropsy found congo red retained in the amyloid tissue in a case in which the dye test had been given during life. Bennhold [8] made the same observation.

In this case, the congo red was prominent one hundred and five days after the first injection and eighty-four days after the second. The addition of dilute hydrochloric acid revealed the presence of the dye by turning it blue. This reaction was reversible in tissue which had been in formaldehyde as long as six months.

16. Bookman, Arthur, and Rosenthal, Julius: Am. J. M. Sc. **173**:396, 1927.

TISSUE REACTION TO COLLOID AND LIPOIDS FROM THE HUMAN THYROID GLAND

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The inflammatory foci with foreign body giant cells found occasionally on microscopic examination of sections of surgically removed human thyroid tissue sometimes have a striking resemblance to the lesions produced by tubercle bacilli. These foci are composed usually of a number of giant cells of the Langhans type, many mononuclear phagocytic cells, a few lymphocytes, a few polymorphonuclear leukocytes and fibroblasts. The fibroblasts are most frequently arranged about the periphery of the focus. Occasionally, a few fibroblasts are arranged radially about the center of the focus. Such foci, especially if they contain a few necrotic cells, may closely resemble the tubercles produced by tubercle bacilli.

These inflammatory lesions vary considerably in appearance. This variation apparently depends partly on their age and partly on the etiologic agent. Foci that are very cellular, contain many giant cells and show only slight to moderate proliferation of fibroblasts are considered to be of short duration. Foci that show extensive sclerosis with an occasional giant cell or without giant cells are thought to represent almost the end-stage of the reaction, with nearly complete fibrosis of the focus. In certain instances, even in the same section of the thyroid tissue, the various gradations between the active cellular reaction and the complete fibrosis of the focus may be seen.

Deposits of cholesterol crystals occur quite often in adenomas of the thyroid gland that show cystic degeneration and in colloid adenomatous goiters. Microscopic examination of these glands not infrequently shows inflammatory foci with foreign body giant cells in relation to the cholesterol crystals. Occasionally, however, inflammatory foci with foreign body giant cells are found which have no relation to cholesterol crystals. These foci usually occur around masses of deeply stained colloid in follicles in which the epithelium is disintegrating. At times there is no visible substance in relation to the focus which may act as an irritant. These reactions are almost always found in thyroid glands that show primary hyperplasia with varying degrees of involution or

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strumitis, and in chronic thyroiditis. However, they are occasionally found in colloid adenomatous goiters. The cause of these inflammatory foci which more or less resemble tubercles is not known.

The fact that such foci often occur in relation to deeply staining inspissated colloid in disintegrating follicles suggests the possibility that the colloid may contain, or may itself be, the etiologic agent. If this is so, it should be possible to produce a similar lesion experimentally by

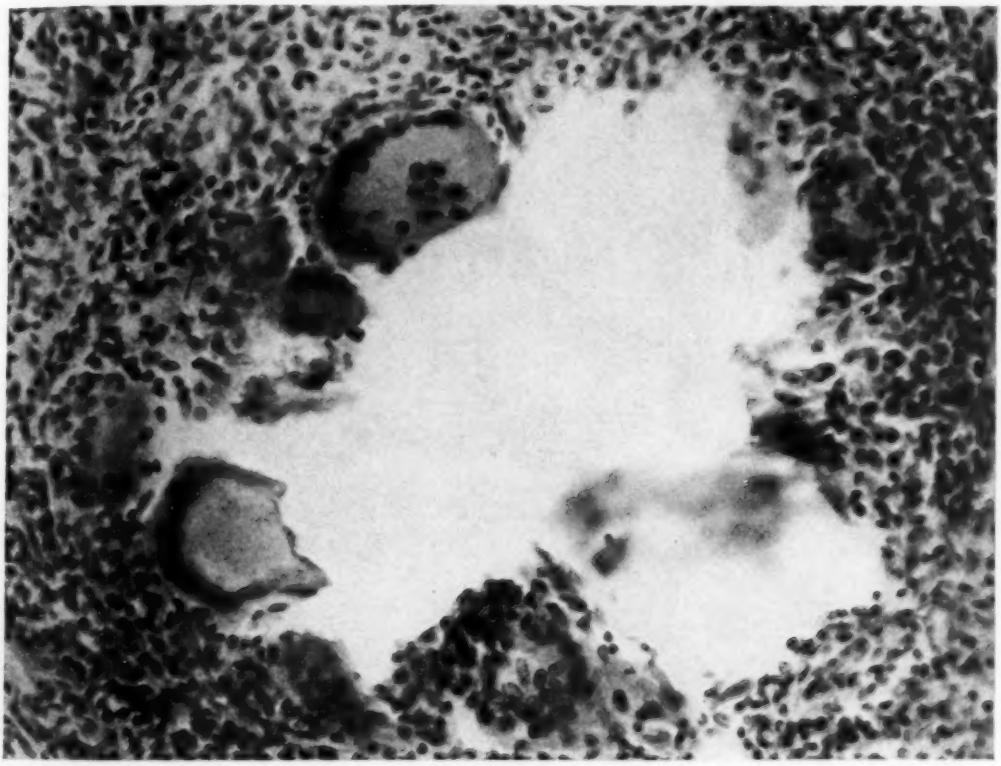

Fig. 1.—Foreign body giant cells around a thyroid follicle from which the epithelium has disappeared; × 150.

means of the colloid itself or by means of substances extracted from the colloid.

The object of this investigation, therefore, is to determine the reaction on connective tissue of sterile dehydrated colloid from the human thyroid gland and of ether and absolute alcohol extracts from the thyroid gland and from the colloid.

MATERIALS AND METHODS

The colloid material used in this investigation was aspirated with a 20 cc. syringe from surgically removed human thyroid glands. The fresh glands of the

colloid adenomatous goiters were selected. Occasionally, 30 cc. of clear amber-colored colloid was withdrawn from colloid cysts, and from 1 to 2 cc. of much thicker material from encapsulated adenomas. The colloid material was centrifugated at high speed to throw down any epithelial cells or blood cells present. The supernatant material was poured into sterile evaporating dishes and evaporated to dryness in an oven at 50 C. The dry material, which was a glassy, brittle substance, was powdered in a sterile mortar and then stored in the icebox until used. Care was taken at each step to avoid contamination.

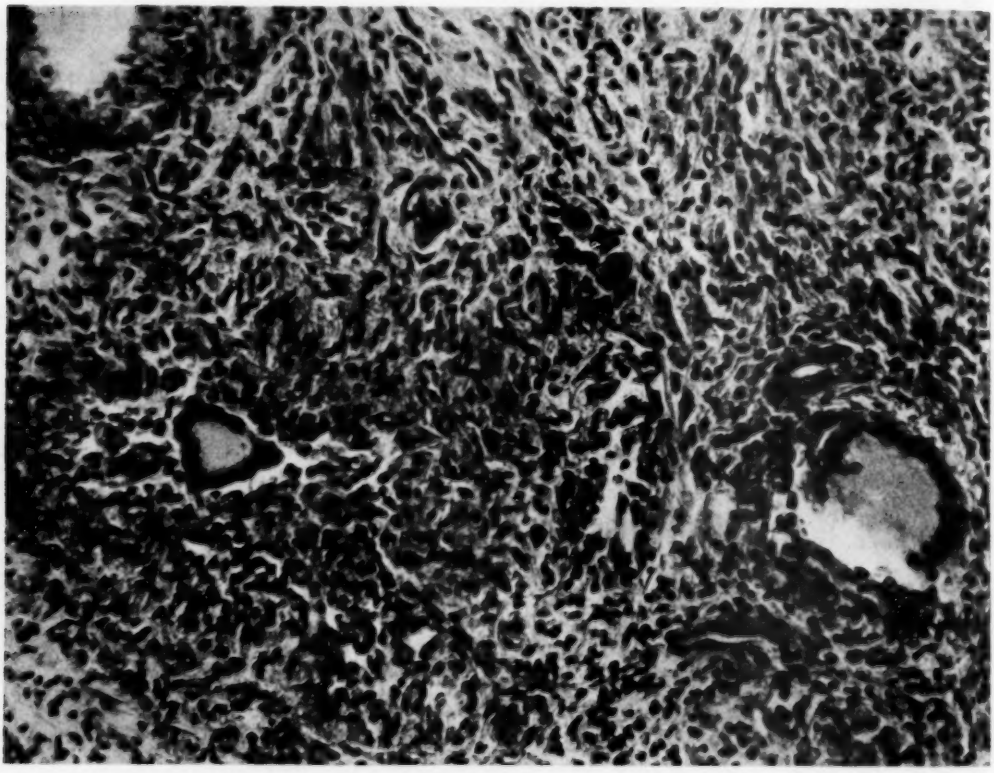

Fig. 2.—Tubercle-like structures in a section of surgically removed human thyroid gland; × 180.

The lipoid material used was extracted from the dehydrated colloid and from the whole thyroid gland. About 4 Gm. of powdered colloid was extracted with ether for one hour and then with absolute alcohol. Each extract and the residue were placed in separate containers. Portions of glands the change in which was diagnosed as primary hyperplasia were minced, washed free from blood and fixed in a diluted solution of formaldehyde, U. S. P. (1:10). After fixation, the material was washed thoroughly and dried. The dry material was extracted with ether in Soxhlet's apparatus for two days and then with absolute alcohol. After the solvent had evaporated, the ether extract appeared yellowish and oily. Needle-like crystals of cholesterol formed on the edge of the extract. The absolute alcohol-

soluble material was brownish and gluelike. No cholesterol crystals formed on the extract, and chemical tests were negative for cholesterol.

For the sake of clearness, this investigation was divided into three experiments. The purpose of experiment 1 was to study the reaction on connective tissue of 1 cc. of thick saline emulsions of the ether extract and of the absolute alcohol extract of the thyroid gland. Experiment 2 was the same, except that the ether and absolute alcohol extracts of the colloid and the colloid residue were used. Experiment 3 was to study the effect on connective tissue of 1 cc. of a saturated saline suspension of dehydrated colloid.

One guinea-pig was used for each experiment. All injections were given subcutaneously in the abdominal region at definite locations, so that the reaction of each substance might be readily distinguished. In experiment 3, eight injections, twenty-four hours apart, were given to the same guinea-pig. The purpose of this procedure was to enable one to study progressively, day by day, the histologic changes that took place in each focus. The material was prepared for microscopic examination by the usual method, and eosin-methylene blue stain was used. In experiments 1 and 2, the guinea-pigs were killed on the sixth day following the injection of the lipoid substances and in experiment 3, the guinea-pig was killed on the eighth day.

PATHOLOGIC OBSERVATIONS

Grossly, the experimental lesions appeared similar. They were slightly raised, rounded nodules, measuring about 0.5 cm. in diameter. The skin was freely movable over the nodules. They showed no signs of infection, and they decreased steadily in size.

Experiment 1.—Microscopic examination of sections from the focus produced by the ether extract and from that produced by the alcoholic extract showed many mononuclear phagocytic cells, some of which contained small masses of lipoid material. The sections showed also many foreign body giant cells, a few polymorphonuclear leukocytes and a few lymphocytes. The giant cells in certain parts of the sections surrounded masses of the injected lipoid material. Throughout the focus, but more particularly at the periphery, there were a great many fibroblasts. An occasional fibroblast was in mitotic division.

Experiment 2.—Microscopic examination of the focus caused by the ether extract of the colloid showed an inflammatory reaction with many mononuclear phagocytic cells, which were phagocytosing the injected material. There were a few lymphocytes, many young fibroblasts and an occasional foreign body giant cell in the focus. The giant cells apparently were just beginning to be formed. The reaction produced by the absolute alcohol extract of the colloid appeared similar, except that there were a few polymorphonuclear leukocytes and a few eosinophils in the focus. The section through the focus caused by the colloid residue showed many mononuclear phagocytic cells and foreign body giant cells formed around the injected material, which was in deep bluish-stained masses.

Experiment 3.—Microscopic examination of sections through the focus of twenty-four hour's duration caused by the saline suspension of the dehydrated colloid showed slight edema of the tissue and infiltration by a few mononuclear phagocytic cells and a few eosinophilic leukocytes. The veins were considerably dilated. Numerous small, deeply stained particles of the injected material were observed. The swelling of the tissue was gone at the end of forty-eight hours. At this time, many new

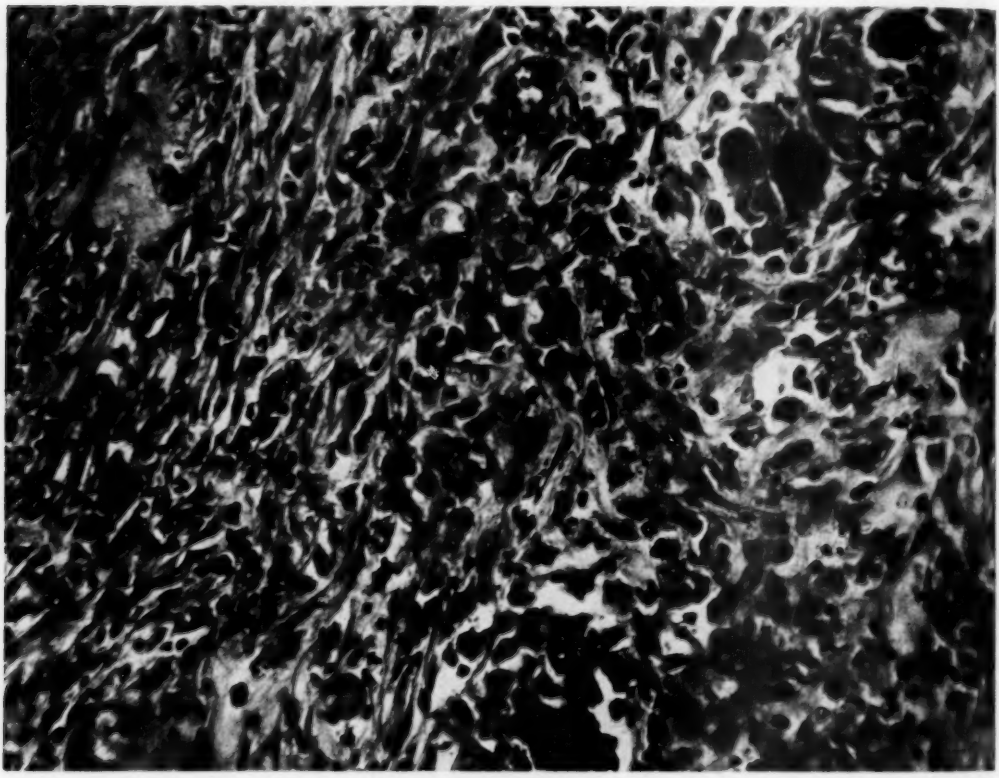

Fig. 3.—An inflammatory reaction with foreign body giant cells in the subcutaneous tissue of a guinea-pig caused by absolute alcohol-soluble lipoids from the thyroid colloid. The fibroblastic proliferation and lack of necrosis are shown; × 200.

fibroblasts had formed, mostly at the periphery of the focus. Some of the smaller veins were thrombosed, and many lymphocytes were collected around them. The lymphatic vessels were dilated. The foci of the third, fourth and fifth days appeared similar. The fibrous tissue was more dense, and many phagocytic cells contained particles of the colloid material. The focus of six days' duration showed foreign body giant cells collected around the colloid particles. There were also a few

polymorphonuclear leukocytes and lymphocytes throughout the focus. In the foci of seven and eight days' duration, most of the phagocytic cells had disappeared, and the foci were almost completely fibrosed.

In summarizing the chief pathologic observations of these three experiments, I find that each reaction was characterized by the formation of foreign body giant cells, marked proliferation of fibroblasts and lack of necrosis.

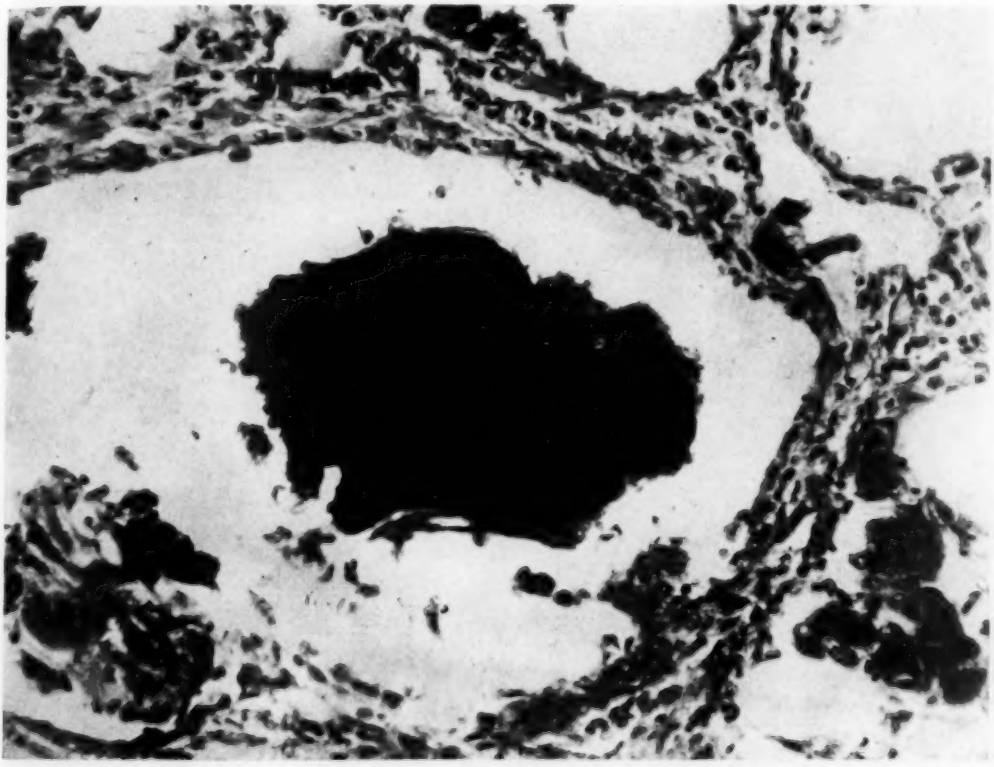

Fig. 4.—The reaction of the subcutaneous tissue of the guinea-pig to dehydrated colloid. Foreign body giant cells are forming around the injected material; × 150.

COMMENT

Lipoid substances are known to be present in the epithelium of the thyroid gland and have been demonstrated to be present in the colloid and stroma.[1] It has been stated that cholesterol may be a constituent of all cells, and that when cells undergo disintegration, cholesterol accumulates as one of the most insoluble products.[2] Cholesterol crystals that

1. Jaffé, R. H.: Histologic Studies on the Fat Content of the Normal Human Thyroids, Arch. Path. **3**:955, 1927.

2. Wells, H. G.: Chemical Pathology, ed. 5, Philadelphia, W. B. Saunders Company, 1925, p. 465.

may be found in tissue under pathologic conditions are removed most commonly by mononuclear phagocytic cells and by foreign body giant cells that form around them. Because cholesterol is readily soluble in ether, the inflammatory reaction with foreign body giant cells produced in the connective tissue of the guinea-pig by ether-soluble material from goitrous thyroid glands is, without doubt, mainly caused by cholesterol, though other lipoids may be present. This inflammatory focus, with

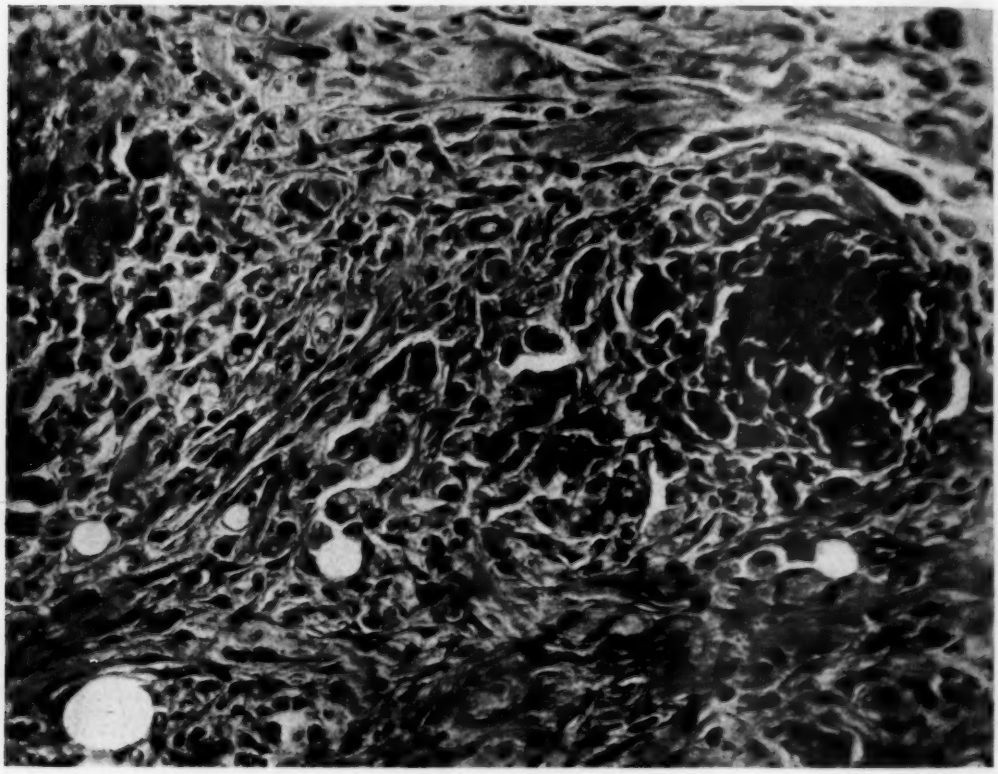

Fig. 5.—The reaction caused by absolute alcohol soluble lipoids from the thyroid in subcutaneous tissue of a guinea-pig; × 300.

the exception that it does not contain cholesterol crystals, resembles closely the lesion found in human thyroid glands, which is caused by cholesterol crystals. The lesion is easily identified in microscopic sections of thyroid glands and is of no particular interest.

The alcoholic extract contained no cholesterol, and in experiment 1 the absence of cholesterol was verified chemically; so, in this instance, the inflammatory reaction with foreign body giant cells could not be due to cholesterol. The lipoids of the thyroid colloid and epithelium, accord-

ing to Jaffe,[3] have the staining reactions of phospholipins. This lipoid material, when injected into the subcutaneous tissue of a guinea-pig, is sufficiently irritating, or soon becomes so, to cause an inflammatory reaction and the formation of foreign body giant cells. The change that takes place in the lipoid material is probably a hydrolysis, since neutral fats do not cause an inflammatory reaction. The hydrolysis of a fat may take place owing to the action of enzymes and moisture,[4] or the

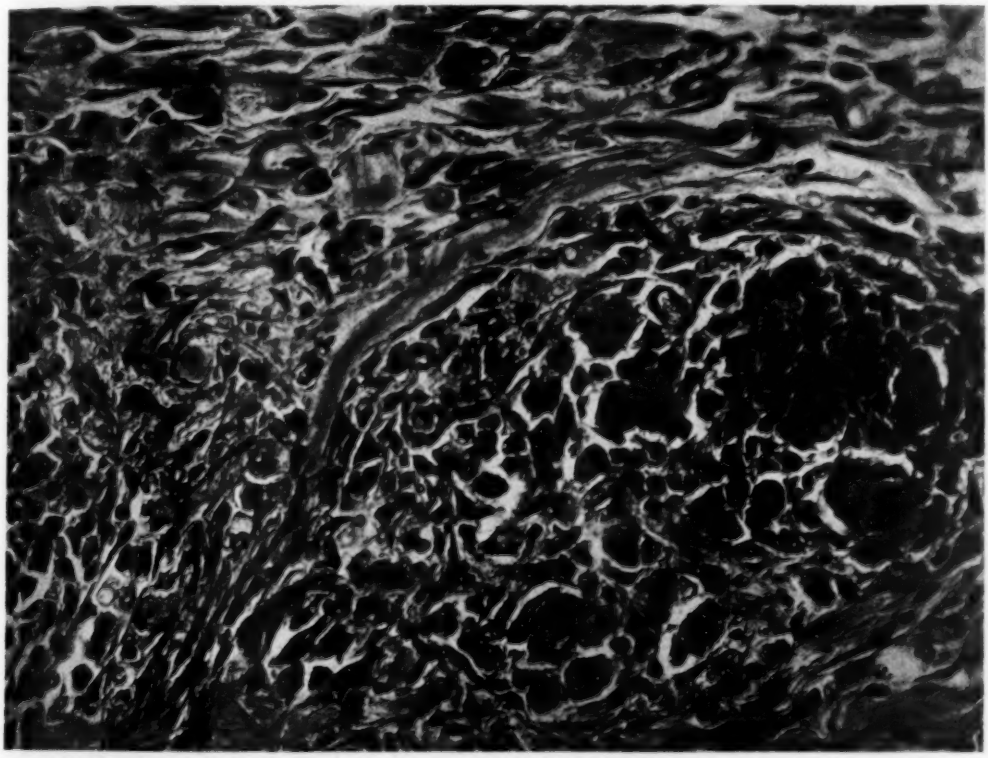

Fig. 6.—The experimental reaction caused by ether-soluble lipoids from the thyroid gland; × 200.

hydrolysis of a glyceride may take place slowly with moisture alone.[5] It seems most likely that the lipoid material undergoes hydrolysis in the

3. Jaffé, R. H.: Tubercle-Like Structures in Human Goiters, Arch. Surg. **21**:717, 1930.

4. Lewkowitsch: Chemical Technology and Analysis of Oils, Fats and Waxes, New York, The Macmillan Company, 1909.

5. Leathes, J. B., and Raper, H. S.: Monographs on Biochemistry: The Fats, ed. 2, New York, Longmans, Green & Co., 1925, p. 140.

subcutaneous tissue of the guinea-pig, the tissue supplying the necessary moisture and enzymes. Thus the liberated fatty acids would readily cause an inflammatory reaction and the formation of foreign body giant cells. Pinkerton [6] concluded from the injection of oils and fats into the trachea of experimental animals, "that the degree of damage and the resulting fibrosis produced by an oil in the lung depends largely on the amount of free fatty acid originally present and the rapidity with which hydrolysis progresses." The inflammatory reaction with foreign body giant cells that may be produced experimentally by means of alcoholic extracts from the thyroid gland and from the colloid is of considerable interest. In the first place, it is not due to cholesterol, but is in all probability due to hydrolysis of the lipoid content of the extract, in the subcutaneous tissue of the guinea-pig. Secondly, the experimental reaction is almost identical with the lesions more or less tubercle-like in appearance that are found occasionally on microscopic examination of thyroid tissue.

These lesions, as has been mentioned, closely resemble tubercles in some instances. This resemblance has been pointed out by Jaffe [3] and others. Tuberculosis of the thyroid gland will not be discussed in this paper, except to observe that Coller and Huggins,[7] Budd and Williams,[8] Jones [9] and others described lesions as tuberculosis of the thyroid gland that appear to be almost identical with the experimental reaction which is due to absolute alcohol-soluble lipoids of the thyroid gland. Budd and Williams stated that tubercle bacilli cannot be demonstrated in sclerosing tuberculosis, and that there is no liquefaction, caseation or necrosis.

It is well known that fats in tissue under certain circumstances cause lesions that resemble those caused by tubercle bacilli. The lipoids of tubercle bacilli may produce lesions that resemble those produced by the organisms.[10] Simonds [11] observed the similarity between lesions caused by experimental suprarenal cell thrombi in the lungs and those caused by tubercle bacilli. Fat necrosis due to trauma at times some-

6. Pinkerton, H.: The Reaction of Oils and Fats in the Lungs, Arch. Path. **5**:380, 1928.

7. Coller, A., and Huggins, C. B.: Tuberculosis of the Thyroid Gland, Ann. Surg. **84**:804, 1926.

8. Budd, S. C., and Williams, C.: Tuberculosis of the Thyroid, J. A. M. A. **92**:1741, 1929.

9. Jones, T. B.: Tuberculosis of the Thyroid, Am. J. Surg. **7**:629, 1929.

10. Auclair, S.: Les poisons du bacille tuberculeux humain, Arch. de méd. exper. et d'anat. path. **7**:629, 1899. MacCallum, W. G.: Textbook of Pathology, ed. 4, Philadelphia, W. B. Saunders Company, 1928, p. 83.

11. Simonds, J. P.: The Organization of Experimental Adrenal Cell Emboli in the Lungs, Am. J. Path. **3**:13, 1927.

what resembles tuberculosis.[12] Because of the close resemblance of many inflammatory lesions with foreign body giant cells caused by lipoid material to the lesions of tuberculosis, the histologic appearance of inflammatory foci which do not show caseation or necrosis and in which tubercle bacilli cannot be demonstrated does not seem to be sufficient evidence for a diagnosis of tuberculosis.

The reaction produced by the lipoid substances in experiment 2 may be explained in a manner similar to that of experiment 1. The only difference is that the lipoid material was extracted from the colloid instead of from the whole gland. The inflammatory reaction with foreign body giant cells caused by the colloid residue in the second experiment is similar to the reaction produced in connective tissue by paraffin, agar-agar and other inert substances.[13] The presence of foreign body giant cells in the lesion of six days' duration in experiment 3 is most probably due to fatty acids resulting from hydrolysis of the lipoids of the injected colloid material.

The fact that phagocytic cells form around colloid in disintegrating follicles in the thyroid gland is evidence that some change is taking place in the colloid. Mallory[14] stated that the colloid probably undergoes some chemical change which causes it to attract endothelial leukocytes into the lumina of the follicles. As a result of this experimental study it appears that the change that takes place in the colloid is a slow hydrolysis of its lipoid content. The fatty acids thus produced would cause the inflammatory reaction without necrosis and with foreign body giant cells that is found occasionally on microscopic examination of human thyroid glands. It seems reasonable to assume that this is so, because almost identical reactions may be produced experimentally in the connective tissue of guinea-pigs by the hydrolysis of lipoids extracted from the whole thyroid gland and from the colloid. Occasionally, the lesions show slight necrosis, but more often it is lacking. The lack of necrosis is due to the slow hydrolysis of the lipoids and the rapid neutralization of the fatty acids formed.

SUMMARY

Colloid and lipoid substances from human thyroid glands cause an inflammatory reaction in the subcutaneous tissue of guinea-pigs. The reaction is characterized by the formation of foreign body giant cells,

12. Lee, B. S., and Adair, F. E.: Traumatic Fat Necrosis of the Female Breast and Its Differentiation from Carcinoma, Ann. Surg. **72**:188, 1920.

13. MacCallum (footnote 10, second reference, p. 208).

14. Mallory, F. B.: Principles of Pathologic Histology, Philadelphia, W. B. Saunders Company, 1918, p. 658.

marked proliferation of fibroblasts and absence of necrosis. The lesion in the tissue of the guinea-pig is apparently caused by the lipoids of the injected material, and it is almost identical with the inflammatory structures which may be tubercle-like in appearance occasionally found on microscopic examination of human thyroid glands.

This experimental study appears to show that such inflammatory structures occurring at times in human thyroid glands are caused by fatty acids. The fatty acids are formed in disintegrating follicles as a result of hydrolysis of the lipoid content of the colloid and of the epithelium. Such reactions are of considerable interest because at times they have a striking resemblance to the lesion produced by the tubercle bacillus.

Laboratory Methods and Technical Notes

CORE DRILL (TREPHINE) FOR POSTMORTEM EXPOSURE OF ACCESSORY NASAL SINUSES, NASAL PASSAGES, MOUTH AND PHARYNX

F. Lowell Dunn, M.D., and D. Rhea
OMAHA

The upper respiratory tract occupies an important place in concepts of portals of entry for infections, yet it is surprising how rarely this region is subjected to study at autopsy. A systematic study of atria of infection would seem to be an essential step in giving direction to present day enthusiasm in preventive medicine. The marked disagreement of able medical men regarding the rôle of head infection in systemic disease becomes intelligible when one searches in vain for published postmortem studies. Although Harke [1] reported 135 instances of gross inflammation of the paranasal sinuses in 399 autopsies, it was not determined whether these findings were etiologic or incidental. There are numerous studies of surgical material and clinical case records, but here one meets the uncertainties and difficulties of interpretation common to statistical methods.

This lack of systematic study of head infections in autopsy material is perhaps a reflection of technical difficulties. The approach to the pharynx, tonsils or paranasal accessory sinuses through the mouth is almost always opposed by the undertaker. After removal of the calvarium and brain, to chip one's way down to the maxillary sinuses and pharynx is a slow task, usually coming at the end of an autopsy that has already consumed its allotted time. The sagittal head-splitting technic described by Harke requires considerable skill, and there are also the cosmetic problems involved in the realinement of the structures.

A technic for studying this region in order to have widespread usefulness must satisfy certain conditions: 1. It must be rapid and simple. 2. The exposure must be ample. 3. The technic should not introduce embalming problems that require unusual skill. A consideration of these requirements led us to the development of a core drill, or trephine, with which one can easily expose the pharynx and paranasal sinuses through the floor of the cranial cavity.

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1. Harke, E. A. T.: Beiträge zur Pathologie und Therapie der oberen Athmungswege einschliesslich des Gehörs, Wiesbaden, J. F. Bergmann, 1895; quoted by Runge, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1928, vol. 3, pt. 1, p. 132.

Figure 1 is a schematic midsagittal section of the head. If a core bounded by the heavy black lines could be removed, there should be a view from above, of the tongue, tonsils, turbinates, back teeth and lower pharynx. With a core drill of sufficient diameter, portions of the medial walls of the maxillary sinuses should be sheared off, permitting their visual examination. At the same time the core removed

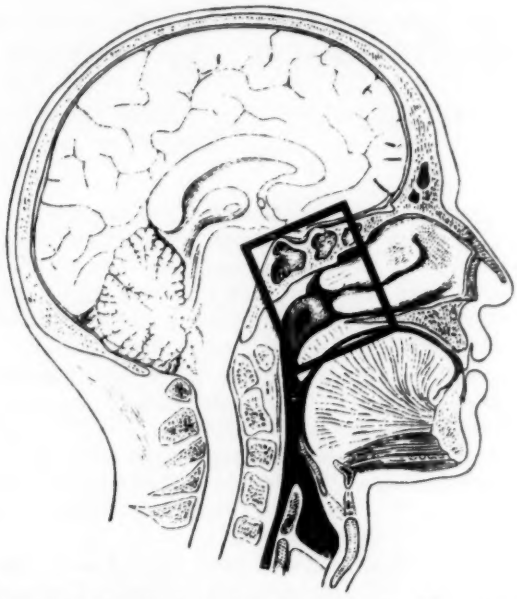

Fig. 1.—Schematic midsagittal section of the head. The area bounded by the black line is the region removed.

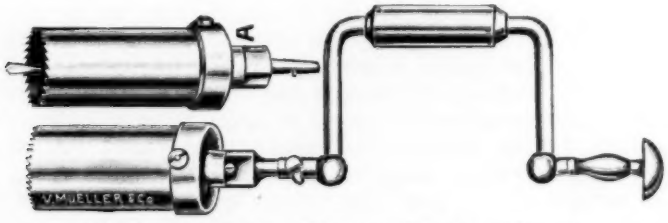

Fig. 2.—Core drill. Diameter 2 inches (5 cm.).

should contain the posterior ethmoid cells, the sphenoids, the posterior nasopharynx, the posterior turbinates and septum, and portions of the hard and soft palate. By this procedure the bony supports of the face are not interfered with, and the orbits are scarcely entered. There is no new break in the circulation, since the internal carotid artery was severed when the brain was removed.

Various sizes of core drills were tried, and that 2 inches (5 cm.) in diameter proved best for the average or even the large adult. This is not the largest size that could be used, but it has the advantage of

not requiring accurate centering, and at the same time it gives an adequate exposure. The axis of the direction of drilling is posterior to the teeth, so that the 2 inch size allows some deviation of the axis without striking the teeth. The extreme hardness of the teeth makes it particularly desirable to avoid them. Smaller sizes are of course necessary for children.

The core drill is illustrated in figure 2. The saw is made of seamless steel tubing with a wall thickness of one sixteenth of an inch (0.16 cm.). The teeth should be coarse, and should number from 6 to 8 to the inch, as finer teeth become clogged. It is not necessary to use any set. The length of the tubing is 4½ inches (11.25 cm.). This seemingly excessive length is required because of the size of the drill and the oblique angle of cutting. The holder for the saw is that used for the metal hole saw in commercial use for cutting into electrical conduit boxes. This type of holder permits removal of the saws for sharpening and replacement, as well as providing a shank to which is fitted a hand brace, and a center drill to steady the saw at the beginning of the cut.

After removal of the calvarium and brain, the pituitary body is enucleated, if it is to be examined. The center drill is then placed in the pituitary fossa and the core drill pointed in the midsagittal plane about midway between the tip and the angle of the mandible. The notch of the facial artery on the mandible is a convenient guide. If the direction is too far posterior, the strong anterior cervical ligaments hold the core tenaciously. It is important that the axis of cutting be in the midsagittal plane, so that the teeth are avoided. The drill chatters for a few revolutions at first, but then takes hold smoothly, and in a few minutes one can feel that it is cutting soft tissue. The drill is then removed. The core is usually still held by soft tissues. However, owing to the thickness of the tubing, a sixteenth of an inch (0.16 cm.) or more of bone has been ground away so that the core can be moved about an eighth of an inch. This permits sliding a thin-bladed knife down through the fissure to cut the soft tissue. Various hook knives suggest themselves, but these have not proved necessary. The core can also be pulled out by traction. We prefer placing a slip noose of twine around the core and wrapping the free ends around a chisel for this traction.

Figure 3 shows the hole made by the drill. It is impossible to demonstrate this exposure effectively by a photograph. The tip of the probe is in the left maxillary sinus, which in this case happened to be unusually small. The tongue is well shown, and because of the large opening it can be examined thoroughly. The tonsils are easily pulled into view and removed. Pressing the base of the tongue exposes the lower pharynx, epiglottis and larynx. The back teeth are visible, but we have made no attempt to develop a suitable plier to remove them.

The core removed is illustrated in figure 4. It has been sawed open in a midsagittal plane. The accessibility is similar to that obtained by the Harke technic mentioned. The convenience in examination of the posterior ethmoids, the sphenoids, the posterior portions of the turbinates, the septum and the palate is apparent. The core includes the nasopharynx. The anterior ethmoids and frontal sinuses must of course be exposed in the usual manner.

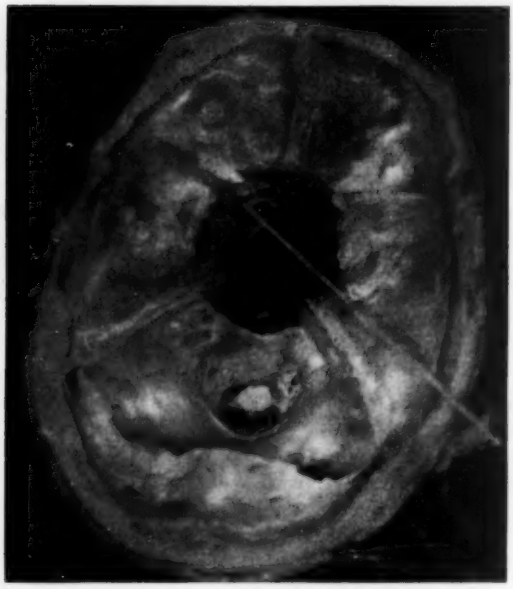

Fig. 3.—Hole after removal of the core. The tip of the probe is in the left maxillary sinus, which in this case is unusually small. The tongue occupies most of the floor. The nasal septum and turbinates are at the top to the right of the probe.

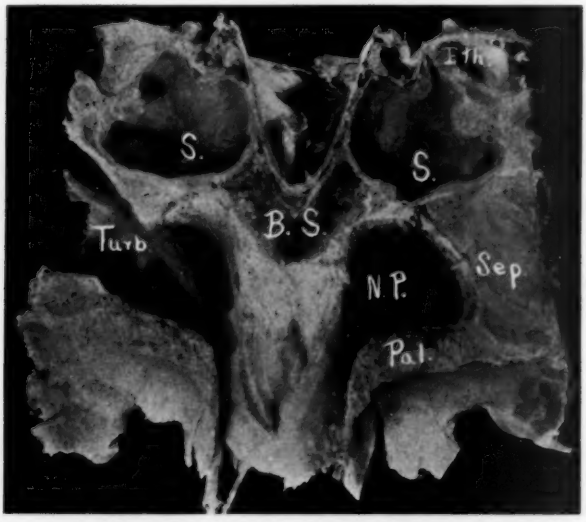

Fig. 4.—The core has been sawed in the midsagittal plane and opened flat with the anterior cervical vertebral fascia as a hinge. *S* designates the sphenoid sinus; *Eth.*, the ethmoid sinus; *B.S.*, the base of the sphenoid; *N.P.*, the nasopharynx; *Sep.*, the nasal septum; *Turb.*, the turbinate, and *Pal.*, the palate.

Whether or not the body is embalmed before the use of the drill is immaterial. The difficulties of embalming after the autopsy are not greater than those involved in the usual head examinations in which the internal carotids are necessarily cut. The leakage of embalming fluid can be prevented by ligating these vessels with a curved needle and holder in the pharynx, or by the usual methods.

SUMMARY

A core drill has been described with which a rapid exposure of the sphenoids, posterior ethmoids and maxillary sinuses, as well as of the tongue, tonsils and pharynx, may be attained.

The need of systematic postmortem study of this region to determine the rôle that head infections may play in lethal disease is emphasized.[2]

2. The core drill may be obtained from V. Mueller and Company, Chicago.

CORRECTION

In the article by Drs. B. I. Phillips, S. J. Newsom, N. W. Jones and Olof Larsell, entitled, "Hematopoietic Effects of Nuclear Extractives Obtained from Red Blood Cells of the Fowl," in the October issue (**14**:498, 1932), the second column in tables 1 to 5, inclusive, should bear the heading "Weight, Lb." instead of "Weight, Kg." Attention is also called to the fact that the name of the fourth author is "Olof" instead of "Oloff."

General Review

THE PROBLEM OF DENTAL CARIES

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Research in dental caries has been so active within recent years that those not directly engaged in it may have been unable to keep pace with its development. More is known about the subject this year than was known last, and it begins to appear that a complete solution may be arrived at before many more years have passed. In this review I have attempted to collect and evaluate the recent developments and to present the subject as it stands at the moment—unfinished, but apparently moving toward a solution.

CLINICAL AND PATHOLOGIC ASPECTS

To provide the background for a discussion of modern research into the problem, one must first review the recognized facts of clinical dentistry. The decay of teeth, in itself and through its immediate and remote sequels, today provides the general practitioner of dentistry with the greater part of his work. At present the condition is so widespread that adults in civilized communities who have never suffered from it are uncommon. Although it appears that the incidence of caries has increased with advancing civilization, its origin is buried in the remote past. It appears to be a peculiarly human disease, although instances of the occurrence of similar lesions in the teeth of dogs,[1] monkeys and other animals [2] have been recorded. It has recently been produced experimentally in rats.

In the human mouth at least two, and possibly more, varieties of tooth "decay" may be distinguished on the basis of definite pathologic differences: (1) a variety which attacks the teeth of young persons, and which occurs either in the pits and fissures of the crown or in the neighborhood of the points of contact of approximating teeth, and

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1. Müller, G., and Glass, A.: Diseases of the Dog and Their Treatment, London, Ballière, Tindall & Cox, 1916.

2. Miller, W. D.: The Micro-Organism of the Human Mouth, Philadelphia, S. S. White, 1890.

which, generally penetrates the enamel with only sharply localized destruction of that tissue, spreads widely in the dentin and approaches the pulp rapidly; (2) one or more varieties which occur in the teeth of adults and the aged, and which commonly attack smooth surfaces of the crown or exposed root, producing a large aperture at the surface and penetrating slowly; and (3) other varieties which occur relatively rarely, including the "odontoclasia" of Simonton and Jones.[3] Of the two main varieties of "decay," the former is so much the more common that, were it the only form of dental caries known, the public health problem involved would hardly be less serious than it is. For this reason, and because the "adult" varieties are sufficiently different from the other to warrant the proviso that their etiology may be distinct, it seems justifiable provisionally to distinguish sharply between them. I shall be concerned here, therefore, chiefly with the first, more prevalent variety; although, since this distinction has not been made by many writers on the subject, a discussion of the literature must to some extent involve all forms indiscriminately. This distinction is of importance particularly in the field of experimental caries.

Dental caries is, then, primarily a disease of children, adolescents and young adults. The period of greatest susceptibility is roughly concomitant with that of eruption of the teeth, and reaches its highest level during the interval of transition from deciduous to permanent dentitions, or shortly thereafter. It is not related, at least obviously, to disease elsewhere in the body; i. e., it occurs in persons who appear to be quite normal otherwise. Statements are occasionally encountered to the effect that the incidence of caries increases in the presence of certain systemic diseases, such as diabetes, but such contentions cannot be regarded as established. It is frequently stated and commonly believed that a rise in the incidence of caries occurs during or as a result of pregnancy, whence the well known maxim "a tooth for every child," but this, too, as the evidence shows, is doubtful. There is also a widespread clinical view that caries involves conditions antagonistic to pyorrhea, since the two diseases seldom occur together; but whether this is not merely coincidence, depending on differences in ages of susceptibility,[4] is uncertain. Much has been made of the relationship of poor oral hygiene to dental caries, and it is probable that careful oral hygiene remains an important factor in prevention; but the phrase "a clean tooth never decays" cannot yet be accepted as fact, at all events in view of the circumstance that the word "clean," thus employed, can have only a relative meaning.

3. Simonton, F. V., and Jones, M. R.: J. Am. Dent. A. **14**:894, 1927.
4. Tischler, B.: Dental Cosmos **71**:579, 1929.

The incidence of dental caries differs widely among various races of mankind; uncivilized races particularly are often free from it (e. g., Maoris,[5] African tribes,[6] Eskimos [7]) when living under "natural" conditions, and develop it when conditions of civilization are introduced. It is likely that change of diet is particularly responsible, but the evidence is conflicting and uncertain as to the precise mechanism involved.

Enamel, the ectodermal tissue covering the crown of the tooth, in which caries begins, is a densely calcified tissue, containing only from 0.3 to 0.5 per cent of organic substance—keratin [8]—disposed as a tenuous reticulum through it, and representing presumably the keratinized remnant of the organic matrix of forming enamel. The enamel is soluble in dilute acids, and when thus dissolved, the keratin, because of its physical delicacy, is mechanically lost, unless special methods are employed to retain it.[9] Dentin, the mesodermal bulk of the tooth, on the other hand, contains about 30 per cent of organic matter [10]—chiefly collagen and elastin. When decalcified, dentin retains its original form and histologic character, appearing as an amorphous or densely fibrous matrix, penetrated by tubules about 2 microns in diameter, extending from the pulp to the inner surface of the enamel. Each tubule is lined with an organic sheath and contains a protoplasmic fibril, a process of an odontoblast, the pulp lining cell. It appears that enamel may be destroyed, for practical purposes completely, through the agency of dilute acids, but that dentin requires for its destruction both demineralization of the inorganic, and digestion of the organic, constituents.

The type of dental caries that occurs in young persons may be regarded as a definite clinical and pathologic entity; it is not merely a "hole in a tooth," nor is any broken or "hollow" tooth necessarily carious. The clinical and pathologic picture of the lesion varies in minor detail with the position of its attack on the tooth surface, but in all cases it is essentially as follows: The lesion first appears clinically as an enlarged developmental pit in the enamel of the crown, or as a chalky, friable, frequently discolored spot in the enamel at the contact point, in either case admitting the end of a fine-pointed instrument. Later, softening and cavitation occur in the underlying dentin, spreading so as to undermine the enamel, in which the aperture may remain small

5. Pickerill, H. P.: The Prevention of Dental Caries and Oral Sepsis, ed. 1, London, Ballière, Tindall & Cox, 1912.

6. Colyer, S.: Dental Rec. **40**:10, 1921.

7. Waugh, L. M.: J. Dent. Research **8**:428, 1928; **10**:387, 1930.

8. Rosebury, T.: J. Dent. Research **10**:187, 1930.

9. Bödecker, C. F.: Dental Rev. **19**:448, 1905; **20**:317, 1906.

10. Bertz: Thesis, Würzburg, 1899, p. 36; cited by Hawk and Bergeim: Practical Physiological Chemistry, ed. 9, Philadelphia, P. Blakiston's Son & Co., 1926.

until overhanging enamel is fractured under the stress of mastication. At this stage, the walls of the "cavity" are generally discolored. Histologically, the lesion may be seen to penetrate the enamel focally, following the direction of the enamel prisms more or less closely, to the dentin. There it spreads laterally, along the junction of dentin and enamel, and then proceeds toward the pulp along the dentinal tubules, producing a characteristic flamelike picture of penetration as a result of the primary curvatures of the tubules. The dentin is first softened, presumably by decalcification, and then undergoes digestion at so-called "liquefaction foci" along the course of the tubules. These foci enlarge and coalesce to form the clinical cavity. The visible process is evidently preceded by advancing bacteria, since the pulp becomes infected before clinical or histologic pulp "exposure" has occurred.[11] When such exposure occurs, pulpitis results, leading invariably, unless treated, to necrosis and gangrene. Alveolar abscess and other root infections may then follow.

It is clear that dental caries, so far at least as the eye can see, begins on the surface of the tooth and progresses inward. The process, as stated, occurs locally at certain points on the tooth surface. These points—the pits and fissures of the crown, and the areas between approximating teeth—are so situated as to be out of contact with opposing teeth in occlusion. They are the regions most likely to accumulate food débris, partly because of their architecture, and partly because they are protected from the normal cleansing movements of food and of the oral tissues in mastication. They are, as well, the regions most difficult to reach with the toothbrush. These facts form the basis of Black's[12] doctrine of extension in preparation of the cavity—a procedure now universally employed. It has been found invariably true that caries does not recur locally when a cavity, after the removal of damaged tissue, is extended beyond the limits of these "out-of-contact" areas to regions that are "self-cleansing," then suitably disinfected, and filled with an inert, durable material placed so as effectively to seal the margins of the cavity. This circumstance is one of the fundamentals of modern dentistry, and its significance in the problem of dental caries cannot profitably be overlooked. A method of preventing dental caries, advanced by Hyatt,[13] involves the operative removal of these "susceptible areas" in the mouths of children, and their replacement with fillings as soon as practicable after the eruption of the teeth. This method, known as "prophylactic odontotomy," is naturally not the desideratum

11. Henrici, A. T., and Hartzell, T. B.: J. Dent. Research **1**:419, 1919.

12. Black, G. V.: A Work on Operative Dentistry, ed. 1, Chicago, Medico-Dental Publishing Company, 1908, vol. 1.

13. Hyatt, T. P.: Dental Items Interest **41**:317, 1925.

one would like to achieve in the prevention of caries, if only because its universal application presents serious practical difficulties; but it has been widely and rather successfully employed.

Dental caries may attack one or several teeth. It occurs most commonly in the molars, next in the upper incisors and in the premolars, and only rarely in the canines and the lower incisors. Its incidence in an individual mouth is frequently, though not always, bilateral. Interdental caries in one tooth generally spreads to the tooth in contact, but does not always do so.

Dental caries is a unique disease: it has no homologue in other tissues or parts of the body. It should be clearly differentiated from caries of bone, otherwise rarefying osteitis, which is an inflammatory condition. There are no inflammatory changes in caries in itself, except those occurring in the pulp as a sequel to it. Enamel and dentin are noncellular tissues, incapable therefore of any cellular reaction to injury; they do not repair themselves or replace lost substance, although additional ("secondary") dentin is generally formed at the expense of existing pulp tissue in response to external irritation, and frequently delays pulp exposure in advancing caries of the dentin.

ETIOLOGY

The doctrine advanced by Miller,[2] sometimes called the chemoparasitic theory of dental caries, which is an explanation of mechanism rather than of cause, has come to be widely accepted. It accords well with the clinical facts which have been given, and in the modified form in which it will be presented here has provided a working hypothesis without a serious competitor. No single fact has yet been elicited to invalidate it; and while it is admittedly incomplete as an explanation of dental caries, its very inadequacy recommends it to the investigator, for it leaves clear and inviting openings for further research.

The Miller doctrine postulates: the retention of suitable carbohydrate food particles at the sites on the tooth surface, where, for the reasons given, such retention may occur; the decomposition of such food by fermentation, brought about by bacteria; the local formation of organic acids, chiefly lactic, in contact with enamel; and the subsequent dissolution of enamel. As I have stated, the enamel may be completely destroyed by the action of acids alone; when the process reaches dentin, however, two phases are necessary—demineralization by acids, and digestion of the organic matrix by putrefying bacteria. The discoloration is assumed to be adventitious. This doctrine has been modified in two important respects. In the first place, "plaques"—organic accretions consisting largely of mucin—may be demonstrated on the tooth surface, particularly in "non self-cleansing" areas; it is

assumed that these plaques may assist the retention of food débris and bacteria, help localize the process, and prevent free access of saliva. Recent evidence [14] to the effect that such plaques, removed from the mouth, are much more freely permeable to dextrose than they are to salivary buffers, contains a further significant suggestion. Secondly, the work of a considerable number of investigators, which I shall describe more fully later, strongly indicates that a single group of micro-organisms—the lactobacilli, related to or identical with L. acidophilus—may alone be responsible for the initiation of caries.

If the foregoing doctrine is accepted provisionally, there are left two questions of outstanding importance, for which Miller's explanation provides no answer: What are the reasons for the varying susceptibility of different persons to dental caries? And how is one to prevent it? Attempts to answer these questions have filled the dental literature in recent years. The result is an array of opinion, hypothesis and fact so extensive that it cannot be reviewed exhaustively here. In general one may distinguish two somewhat conflicting basic concepts: that which considers caries as a manifestation of some systemic disorder, and that which considers it a purely local process; and it will be convenient to consider these separately, although to some extent they overlap.

THE SYSTEMIC APPROACH

It will suffice here to consider only such hypotheses as have been advanced recently, or are now current, and the evidence relating to them. Many older theories have ceased to have more than historical interest. Theories of susceptibility to dental caries which involve a systemic or a metabolic factor generally presuppose one or both of two agencies—variations in the composition of the saliva, and agencies operating through the tooth itself from within.

That the saliva may be potentially capable of neutralizing acids formed at the tooth surface, and thus of preventing caries, has long been recognized, and a large number of workers have endeavored to ascertain the variations in salivary composition and their underlying causes. For the most part the findings have been negative, and have served chiefly to clear the ground; these I shall not discuss. Two theories are now current in this connection, both advanced by British authors, but both finding some support in this country. Broderick,[15] in an extensive theory ramifying beyond dental caries to include many

14. Dobbs, E. C.: J. Dent. Research **12**:445, 1932.

15. Broderick, F. W.: Dental Medicine, St. Louis, C. V. Mosby Company, 1928.

other diseases, assumed that the acid-base balance of the blood varies sufficiently in different persons to produce, in those susceptible to caries, a saliva low in base and hence in neutralizing power, and in non-susceptible persons, the reverse. This author used the terms "acidosis" and "alkalosis" in connection with this theory, but since these terms represent clinical entities to the internist—diseases with which caries certainly need not be associated—the terms are misleading. Evidence to support this view has been advanced by Hawkins,[16] on the basis of extensive determinations of the p_H of saliva and blood, but his work has failed of confirmation in the hands of somewhat more careful workers (Karshan, Krasnow and Krejci;[17] Youngburg[18]). These workers have shown that salivary buffer capacity and p_H do not vary correlatively with the incidence of caries. In general it is now probable that even the most acid saliva has sufficient buffer capacity to make it potentially capable of neutralizing local acids, if it can but gain access to the region of their production. The clinical studies of Jones et al.[19] support Broderick's contention to some extent, but since this worker was dealing with a sporadic rampant type of decay, "odontoclasia," admittedly different from dental caries, her work is perhaps not entirely relevant here. Lennox[20] advanced a view somewhat similar to that of Broderick, based on clinical observation rather than experiment, postulating the agency of a dietary deficiency of phosphorus, which "causes some change to take place in the saliva, that removes its normal inhibiting action." This was supported by Klein and McCollum[21] in a paper based on animal experimentation and on analysis of other papers. These workers have since[22] modified their views considerably, however; and since they seem to have been working with a type of animal tooth decay distinct from dental caries, their conclusions in this regard are subject to reservation.

A theory involving the agency of the tooth pulp, reflecting systemic disturbance, in susceptibility to caries, has been advanced by Bödecker.[23] This view again depends on the agency of the blood buffers, but assumes their action as one of diffusion from the pulp through the dentin and

16. Hawkins, H. F.: J. Am. Dent. A. **16**:781, 1929; J. Dent. Research **11**:201, 1931.

17. Karshan, M.; Krasnow, F., and Krejci, L. E.: J. Dent. Research **11**:573, 1931.

18. Youngburg, G. E.: J. Dent. Research **12**:267, 1932.

19. Jones, M. R.; Larsen, N. P., and Pritchard, G. P.: Dental Cosmos **72**: 439, 574, 685 and 797, 1930.

20. Lennox, J.: Brit. Dent. J. **52**: 1 570, 1931.

21. Klein, H., and McCollum, E. V.: Science **74**:662, 1931.

22. Klein, H., and McCollum, E. V.: J. Dent. Research **12**:524, 1932.

23. Bödecker, C. F.: Dental Cosmos **71**:586, 1929.

enamel to the tooth surface, there neutralizing locally formed acid. Differences are involved between susceptible and caries-free persons either in blood buffer capacity, in pulp activity, or in permeability of the dental tissues. In the first case, the more reliable evidence (Karshan et al.[17]) makes it unlikely that a difference in blood buffer capacity can be correlated with the incidence of caries. In the second, recent experiments on animals suggest to Blackberg and Berke [24] that structural pulp changes may be an important predisposing cause of caries; but a careful study of this work fails to reveal evidence which clearly warrants such opinion. It has recently been found (Rosebury, Karshan and Foley [25]), on the other hand, that caries may occur in the rat in the presence of a histologically normal pulp. In the third case, the dental tissues have been shown to be permeable (Livingston,[26] Klein and Amberson,[27] others) to inorganic radicals if not to larger molecules, but whether such permeability varies correlatively with susceptibility to caries remains to be determined. It is worthy of note that the permeability of the teeth, as well as the activity of the pulp, decreases with advancing age (Beust,[28] others); while susceptibility to dental caries decreases likewise.

A considerable body of work, in addition to that mentioned, has been based on the systemic approach to this problem, without however assuming a completed hypothesis. Much of this work will undoubtedly prove useful in constructing the final story of dental caries. The influence of pregnancy on caries, for example, has been rather extensively studied. There is no doubt that pregnancy may induce structural changes in the teeth of both mother and young when the diet is deficient (Toverud,[29] Macomber,[30] Rosebury and Foley [31]), at least in the case of experimental animals. But the view that such changes necessarily predispose to caries (Mellanby,[32] many others) has not been verified. There is evidence to indicate that structural defects in the teeth of susceptible animals need not be associated with caries (Rosebury and Foley,[33] Klein and McCollum [22]). That an increase in the incidence of caries may occur in pregnant women is indicated by the clinical studies of

24. Blackberg, S. N., and Berke, J. D.: J. Dent. Research **12**:349 and 609, 1932.
25. Rosebury, T.; Karshan, M., and Foley, G.: Unpublished data.
26. Livingston, A.: Proc. Roy. Soc. Med. (Sect. Odont.) **18**:27, 1925.
27. Klein, H., and Amberson, W. R.: J. Dent. Research **9**:667, 1929.
28. Beust, T.: Dental Cosmos **74**:541, 1932.
29. Toverud, G.: Dental Cosmos **69**:1213, 1927.
30. Macomber, D.: J. A. M. A. **88**:6, 1927.
31. Rosebury, T., and Foley, G.: J. Dent. Research, to be published.
32. Mellanby, M.: Brit. Dent. J. **48**:95, 1927.
33. Rosebury, T., and Foley, G.: J. Dent. Research **12**:462, 1932.

Gerson,[34] and generally believed; but the more extensive studies of Ziskin [35] fail to demonstrate this. At all events there is no evidence available to indicate that pregnancy in itself induces caries. It may be that the factor determining an increase of dental caries during or after pregnancy, in isolated cases among women, is included among the multifarious circumstances incidental and adventitious to the period of pregnancy and lactation, rather than to these states themselves.

The possible agency in dental caries of the vitamin deficiency diseases has been prominently discussed in recent years. The most frequently mentioned of these is rickets or, more specifically, a deficiency of some part of the calcium-phosphorus-vitamin D complex. One theory depending on phosphorus deficiency has already been mentioned.[20] Other studies have been based generally on the concept that structural dental defects are related to dental caries. It is now recognized that the teeth are structurally very sensitive to nutritional disturbances of a rachitic character,[36] probably even more sensitive than is bone. Mellanby [32] has been prominent in advancing the view that such defects predispose to caries, and McCollum, Simmonds, Kinney and Grieves [36c] and Blackberg and Berke [24] have produced decay of the teeth of rats and attributed it to rachitic deficiency. The latter work suffers, however, from the defects of inadequate control, and both groups of workers failed to distinguish between true caries and other destructive dental lesions. In this connection certain extensive clinical investigations are of importance. The work of Mellanby and Pattison,[37] Boyd and Drain,[38] Bunting and his associates,[39] and recently McBeath [40] all involved studies of the incidence of caries in groups of children placed on regulated diets. In each case, a reduction of caries, varying from "slight but definite" in the case of Mellanby and Pattison, to nearly total in the case of Boyd and Drain, was obtained with adequate as compared with unregulated diets. Each of these authors stressed the importance of rachitic deficiency. Yet their results do not clearly indi-

34. Gerson, F.: Zahntechnische Rundschau **3**:3, 1921; abstr., Internat. J. Orthodontia **7**:459, 1921.

35. Ziskin, D. E.: Am. J. Obst. & Gynec. **12**:710, 1926.

36. (*a*) Mellanby, M.: Physiol. Rev. **8**:545, 1928; (*b*) Medical Research Council, Special Report Series, no. 153, London, His Majesty's Stationery Office, 1930. (*c*) Grieves, C. J.: J. Am. Dent. A. **9**:467, 1922; **10**:573, 1923. McCollum, E. V.; Simmonds, N.; Kinney, E. M., and Grieves, C. J.: Bull. Johns Hopkins Hosp. **33**:202, 1922.

37. Mellanby, M., and Pattison, C. L.: Brit. M. J. **2**:1079, 1928.

38. Boyd, J. D., and Drain, C. L.: J. A. M. A. **90**:1867, 1928; Boyd, J. D.; Drain, C. L., and Nelson, M. V.: Am. J. Dis. Child. **38**:721, 1929.

39. Bunting, R. W.: J. Dent. Research **9**:291, 1929. Bunting, R. W.; Delves, E. A., and Hard, D. G.: ibid. **10**:374, 1930.

40. McBeath, E. C.: J. Dent. Research **12**:723, 1932; also unpublished data by permission.

cate that this is the important factor. Mellanby and Pattison, and McBeath, alone of these workers, tested the effect of adding only vitamin D (viosterol) to a standard diet, and comparing this with the effect of the same diet without vitamin D. The first workers reported "slight but definite" reduction of caries in the group receiving the vitamin. McBeath found little reduction of caries in this group, although he found greater reduction in a group of children irradiated with ultraviolet rays, and, as reported by Boyd and Drain, and Bunting, groups receiving diets modified in several respects showed less caries than "control" diet groups. It is difficult to interpret these findings, as McBeath pointed out, but the evidence does not seem to warrant the conclusion that vitamin D is the determining factor in susceptibility to caries. On the other hand, these experiments do indicate clearly enough that the incidence of dental caries may be reduced by suitable regulation of the diet. Furthermore, there is evidence of another type which may lead us to doubt the importance of rachitic deficiency in caries. For one thing, a number of workers [41] have reported failures to produce dental caries in rats on diets involving various rachitic deficiencies. Since, as we shall see, the rat has been shown to be susceptible to dental caries, these findings may be significant. Finally Mackay and Rose [42] and Hess and Abramson,[43] investigating the relationship of obvious rickets in children to the incidence of dental caries, failed to observe any correlation.

Much has also been made of the importance of vitamin C deficiency in susceptibility to caries. Analogously to the work on the influence of vitamin D, the work of Zilva and Wells,[44] Höjer [45] and others clearly shows that vitamin C deficiency produces profound changes in dental structure. The extensive experiments of Howe [46] on animals, however, first focused attention on this deficiency as a contributing cause of dental caries. But Howe (1921) produced a generalized softening of all the teeth in guinea-pigs on scorbutic diets—clearly not dental caries; yet he concluded that vitamin C deficiency may be important in human caries. In his work with monkeys, furthermore, Howe (1924) obtained apparently typical caries on diets some of which were without vitamin C and others of which contained large doses of orange juice. He never-

41. (*a*) Knowlton, G. C.: Proc. Soc. Exper. Biol. & Med. **27**:757, 1930. (*b*) Rosebury, T., and Karshan, M.: J. Dent. Research **11**:121 and 137, 1931. (*c*) Lilly, C. A.: J. Nutrition **5**:175, 1932.

42. Mackay, H. M. M., and Rose, S. F.: Lancet **2**:1230, 1931.

43. Hess, A. F., and Abramson, H.: Dental Cosmos **73**:849, 1931.

44. Zilva, S. S., and Wells, F. M.: Proc. Roy. Soc., London, s. B, **90**:505, 1919.

45. Höjer, J. A.: Acta pædiat. (supp.) **3**:39, 1924.

46. Howe, P. R.: Dental Cosmos **62**:586 and 921, 1920; J. Dent. Research **3**:7, 1921; J. Am. Dent. A. **11**:1149, 1924.

theless continued to stress the importance of vitamin C deficiency. This work was applied by Hanke [47] to clinical experimentation with the feeding of orange juice. As a result of his earlier experiments (1929, 1930) Hanke claimed remarkable results for this form of treatment, but he has subsequently (1932) modified his views and joined the other workers engaged in child feeding experiments in advocating a generally well-balanced diet for the reduction of caries in children. It may be noted further in this connection, that, although the teeth (of susceptible animals) are very sensitive to vitamin C deficiency, a fact which forms the basis of present methods of assay of the vitamin,[48] and although dental changes in human scurvy appear to be characteristic,[49] carious human teeth do not show scorbutic changes. If one accepts Hanke's statement that the feeding of orange juice prevents caries, this may still be due, as Hatton [50] has pointed out, to the influence of some ingredient in this complex mixture other than vitamin C. Finally, the work of Westin [49] (cf. also Dalldorf [51] indicated that caries is not increased in clinical scurvy.

The possible agency of other vitamins in preventing caries has not been thoroughly studied. Grieves [36c] and Marshall [52] reported the production of decay in rats on vitamin A-deficient diets. Marshall stated that the lesion simulated caries in adult man, and that produced by Grieves was probably similar. Bloch [53] studied exophthalmia in children, and failed to find the disease correlated with an increased incidence of caries. Finally, in a recent report, Kellogg and Eddy [54] suggested the possible agency of vitamin B deficiency in the production of caries in rats, in conjunction with a diet of low p_H; but their experimental data are rather meager, and the lesion produced [55] is obviously not comparable to human caries.

The recent reports of Hoppert, Webber and Canniff [56] and current experiments in my laboratory,[25] which are discussed more fully in later paragraphs, indicate that dental caries may be produced in rats fed suitable adequate diets, and which are normal in growth and appearance.

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47. Hanke, M. T.: J. Am. Dent. A. **16**:2263, 1929; **17**:957, 1930; J. Dent. Research **12**:518, 1932.

48. Goettsch, M.: Quart. J. Pharm. & Allied Sc. **1**:168, 1928. Eddy, W. H.: Am. J. Pub. Health **19**:1309, 1929.

49. Westin: Ueber Zahn Veränderungen in Fällen von Skorbut bei Homo, Stockholm, A.-B. Fahlcrantz Boktryckeri, 1931.

50. Hatton, E. H.: J. Dent. Research **12**:386, 1932.

51. Dalldorf, G.: Dental Cosmos **74**:700, 1932.

52. Marshall, J. A.: J. Am. Dent. A. **14**:3, 1927.

53. Bloch, C. E.: Acta pædiat. **11**:535, 1930.

54. Kellogg, M., and Eddy, W. H.: Dental Cosmos **74**:334, 1932.

55. Bödecker, C. F., and Applebaum, E.: Dental Cosmos **74**:335, 1932.

56. Hoppert, C. A.; Webber, P. A., and Canniff, T. L.: Science **74**:77, 1931; J. Dent. Research **12**:161, 1932.

Although there is some as yet doubtful evidence that dietary deficiency may increase the incidence of caries or accelerate it in these animals, it now appears clear that such deficiency is not a determining factor in the production of this lesion. This accords well, it may be observed, with the findings of the workers engaged in child-feeding experiments, all of whom obtained subtotal reduction of caries on adequate diets. One is thus left with but a single reliable conclusion from studies based on the systemic approach: that diet, in some manner apparently not depending on the deficiency of any ingredient, plays a definite, perhaps a determining, part in susceptibility to dental caries, and that the incidence of caries in children may be reduced by regulation of the diet. It seems unwise as yet to make this statement more specific.

THE LOCAL APPROACH

The Miller [2] doctrine postulated only conditions operating locally at the surface of the teeth, and left open the possibility that the completed story of dental caries may likewise involve only local factors. This approach to the problem has attracted many workers, and has on the whole been more productive of fact than the systemic approach.

One of the popular concepts which has been rather incompletely investigated is that which relates to varying resistance of the teeth to the caries attack. Pickerill [5] early classified teeth as "sclerotic" and "malacotic," and considered the latter more susceptible to dental caries. Statements relating to "soft teeth, likely to decay," are frequently heard in dental practice and elsewhere; yet evidence supporting such a concept is wanting. Clinically it is certain that "hard" teeth may decay; it is likely that "soft" teeth may decay more rapidly than others, but whether the structure of a tooth is of any real importance in determining whether caries will or will not attack that tooth is doubtful. The views of Mellanby in this connection have already been discussed. I may add here that, although this worker emphasizes the relationship of defects in the enamel to dental caries, other opinions have it (e. g., Bödecker's [23]) that clinically hypoplastic teeth are, in general, no more susceptible to caries than apparently normal teeth.

The question of the influence of diet on caries has attracted workers in the "local" school as well as in the other. Two groups may be discerned here. The first of these has emphasized the agency of a high carbohydrate diet, acting directly by providing pabulum for fermenting bacteria. The possibly deleterious effect of "sweets" on the teeth of children has, indeed, been widely considered. Bunting [57] found that

57. Bunting, R. W.: Dental Cosmos **67**:771, 1925; personal communication, 1930.

rats fed an adequate diet with added "fudge" developed caries, and Shibata,[58] who was the first to report true dental caries in young rats, attributed the lesion to the carbohydrate in the diet. There is evidence to indicate, on the other hand, that a high carbohydrate diet, containing fermentable sugar (dextrose) and starch, need not produce caries in animals.[59] Except for the work of Shibata, which is rather inconclusive on this point, the possible influence of the kind of carbohydrate present in the diet has not been investigated. Obviously carbohydrate cannot be excluded from the diet. Realizing this, and apparently unaware of some of the evidence that had been given, Shibata [60] concluded that dental caries is inherently unpreventable, and recommended the "prophylactic odontotomy" previously mentioned.

The possibility of a direct influence of the physical character of the diet on the production of caries has been suggested by Wallace.[61] Basing his concept on the increased incidence of caries in civilized as compared with savage races, and on a comparison of the diets of the two, he advanced the view that caries results from the soft, cooked diet of civilization, which lacks the detergent action on the teeth of the coarse, more fibrous "natural" diets. Experiments conducted on men (convicts, subject to rather careful control) by Howitt, Fleming and Simonton [62] to test this premise were rather disappointing. These workers found that a so-called "sticky" diet left the teeth cleaner and produced no more abundant bacterial growth in the oral débris, than supposedly detergent diets. This question was recently revived, although in another form, by the work of Hoppert, Webber and Canniff,[56] who reported the production of caries in rats fed adequate diets, and were able to correlate the occurrence of the lesion with the particle size of corn and other cereals in the diet. The caries-producing diets were, however, those containing the coarser particles. Similar diets with finely ground cereal particles failed to produce caries. These findings have been confirmed, to some degree at least, in my laboratory.[25] The work of Hoppert et al.[56] was criticized by Klein and McCollum,[21] on the ground that the cereal particles of different size also differed in phosphorus content; but evidence contained in the later of Hoppert's reports (1932), and the evidence obtained by myself, indicate that varying phosphorus content is not a factor in the production of this lesion. It now seems

58. Shibata, M.: Japan J. Exper. Med. **7**:247, 1929; Shikwa Shimpo, April, 1929, vol. 22.

59. Rosebury and Karshan.[41b] Lilly.[41c] Howe.[46]

60. Shibata, M.: Tr. Nippon Dent. A. 1st Gen. Conf., 1930, p. 123; J. Nippon Dent. A. **23**:214, 1930.

61. Wallace, J. S.: Lancet **2**:218, 1927.

62. Howitt, B. F.; Fleming, W. C., and Simonton, F. V.: Dental Cosmos **70**:575, 1928.

likely that dental caries may be produced and prevented in rats by varying only the cereal particle size from coarse to fine; whether and how this finding can be applied to human caries remains undetermined.

It is necessary at this point to digress briefly in order to discuss the general question of experimental dental caries in rats. I [63] recently reported briefly on a study of the literature in this field, from which it appeared that most of the dental lesions which had been described in rats could not properly be spoken of as caries. There has been a tendency among dental investigators to apply the term "caries" to any destructive dental lesion, except those obviously due only to mechanical abrasion or chemical erosion, and there can be no doubt that the character of the lesions described in the teeth of rats has varied very widely. It has seemed justifiable to me to follow the classification given earlier in this paper, and, provisionally at all events, to reserve the term "caries"—particularly when applying it to findings in animals—only to such lesions as closely resemble the dental caries previously described as a clinical and pathologic entity. This disease is not only a well defined unit, but a major dental problem in itself as well, while other types of dental decay constitute a varied group of less practical importance. Accordingly I suggested that, if studies in rats are to have any application in human caries, three criteria for the recognition of true caries be established: (These are features of differentiation from other lesions, rather than a complete description. It is assumed that the histologic picture must simulate that in human teeth.) The lesion must (1) begin in enamel, (2) occur in young animals, and (3) progress with relative rapidity. Lesions fulfilling these criteria were produced in rats by Shibata [58] in 1929, and have since been produced by Rosebury, Karshan and Foley [64] as a confirmation and extension of Shibata's work. In addition, some at least of the lesions described by Hoppert, Webber and Canniff [56] probably belong in this class. Bunting [65] obtained a lesion beginning in enamel, but occurring in adult animals, and Blackberg and Berke [24] presented a photomicrograph of a lesion in which enamel is involved, but apparently secondarily. All other rat tooth lesions reported with which I am familiar are either definitely distinct or incompletely described. Most of these are lesions which began in dentin, generally at the summit of the molar cusp, where dentin is normally exposed in the rat, and most occurred in adult animals only, and seemed to follow a chronic course.

The production in rats of a lesion closely simulating dental caries of the most prevalent human type opens up a most promising field for

63. Rosebury, T.: J. Dent. Research **12**:255, 1932.
64. Rosebury, T.; Karshan, M., and Foley, G.: J. Dent. Research **12**:464, 1932.
65. Bunting, R. W.: Personal communication, 1932.

experimental research. At present indications from such work in relation to etiology suggest that dental caries in rats depends on the physical character of the diet, with rachitic or other deficiency perhaps an augmenting or accelerating factor; but the findings are as yet too recent and too incomplete to warrant their unquestioned acceptance.

THE BACTERIOLOGY OF DENTAL CARIES

I reserve a separate section for this branch of the local approach to the problem because it is in this field that some of the most striking results in experimental research in dental caries have been obtained. Miller [2] originally based his doctrine largely on his own bacteriologic studies. He found many bacteria in decayed teeth, and pointed to bacterial action as an important feature in caries. Subsequently a series of investigators substantially confirmed this finding, and developed the view that the process is not bacteriologically specific, but might be initiated by any of a group of fermenting bacteria. This work is well reviewed by Appleton; [66] I may pass over it here. Goadby,[67] in 1903, had isolated a gram-positive, nonsporing rod, which he named B. necrodentalis, and which was probably identical with the lactobacilli later accorded prominence; but he did not attach unique significance to it. Kligler and Gies,[68] in 1915, reported the results of extensive quantitative studies of the flora of caries, and emphasized the occurrence of B. acidophilus and thread forms, and the great increase in numbers of these organisms with the development of caries. Many other bacteria were, however, obtained as well. In 1917, Howe and Gillette [69] confirmed these findings, and later Howe and Hatch [70] reported that the so-called Moro-Tissier group of bacteria—B. acidophilus, B. bifidus and others—preponderated in dental caries. With the development of the principle of p_H, McIntosh and his co-workers,[71] and Rodriguez,[72] independently were able to demonstrate that acidophilus-like organisms are the only forms that develop consistently in cultures from carious teeth in a carbohydrate broth of p_H 5. These workers also considered that the acid-producing powers of these organisms were sufficient to decalcify enamel, and concluded that they had found the bacterial cause of dental

66. Appleton, J. L. T., Jr.: Bacterial Infection, Philadelphia, Lea & Febiger, 1925.

67. Goadby, K. W.: The Mycology of the Mouth, New York, Longmans, Green & Co., 1903.

68. Kligler, I. J., and Gies, W. J.: J. Allied Dent. Soc. **10**:141, 282 and 445, 1915.

69. Howe, P. R., and Gillette, H. H.: J. Nat. Dent. A. **4**:997, 1917.

70. Howe, P. R., and Hatch, R. E.: J. M. Research **36**:481, 1917.

71. McIntosh, J.; James, W. W., and Lazarus-Barlow, P.: Brit. J. Exper. Path. **3**:138, 1922; **5**:175, 1924; **6**:260, 1925.

72. Rodriguez, F. E.: Mil. Dent. J. **4**:199, 1922.

caries. The work of Bunting and his collaborators in 1925 and since [73] greatly extended these findings. These investigators showed that lactobacilli are uniformly present in carious teeth, that they are frequently absent from noncarious mouths, and (Jay and Voorhees [74]) that their presence in a noncarious mouth may foreshadow the appearance of clinical caries on later examination. Their findings have been confirmed by several investigators.[75] In the same period, certain English workers [76] reported the isolation of an anhemolytic streptococcus from dental caries, called by them S. mutans, because of its capacity for assuming elongated, rodlike forms, and claimed that it may be distinguished from L. acidophilus on cultural and serologic grounds, and considered it more important than L. acidophilus in the causation of caries. Whatever the value of this opinion may be, it is clear that true streptococci are found only rarely in cultures in mediums of p_H 5 by workers in this country. Bunting considered S. mutans to be a variant of L. acidophilus, which shows coccic forms frequently, and which is culturally and serologically heterogeneous. Nevertheless, allusions to S. mutans in this relation are still encountered in British literature.[77]

Bunting, on the strength of the results given, originally felt justified in concluding that dental caries is a specific infectious disease, with L. acidophilus as etiologic agent. Although one may admit the constant presence of lactobacilli in the lesions, their frequent absence from caries-free mouths, and their unique and apparently significant acidogenic and "aciduric" properties, there are nevertheless serious objections to this view. The organism may often be obtained from mouths without caries, although reduced in numbers [78] or in acid resistance [79] or both. Bunting's own work [73] indicates that it may be fed to caries-free persons in the form of massive doses of milk cultures, without producing caries; and several workers have fed it to animals [59] without effect.

73. Bunting, R. W., and Palmerlee, F.: J. Am. Dent. A. **12**:381, 1925. Bunting, R. W.; Nickerson, G., and Hard, D. G.: Dental Cosmos **68**:931, 1926. Bunting, R. W.; Crowley, M.; Hard, D. G., and Keller, M.: ibid. **70**:1002, 1928. Bunting, R. W.; Nickerson, G.; Hard, D. G., and Crowley, M.: ibid. **70**:1, 1928.

74. Jay, P., and Voorhees, R. S.: Dental Cosmos **69**:977, 1927.

75. (*a*) Thompson, R.: Proc. Soc. Exper. Biol. & Med. **29**:103, 1931. (*b*) Enright, J. J.; Friesell, H. E., and Trescher, M. O.: J. Dent. Research **12**:759, 1932.

76. Clarke, J. K.: Brit. J. Exper. Path. **5**:141, 1924. Maclean, I. H.: Proc. Roy. Soc. Med. (Sec. Odont.) **20**:873, 1927

77. Thomson, D., and Thomson, R.: Ann. Pickett-Thomson Research Lab. **3**:174, 1927; **5**:5, 1929. Fish, E. W.: Dental Surgeon **29**:267, 1932.

78. (*a*) Rodriguez, F. E.: J. Am. Dent. A. **18**:2118, 1931. (*b*) Jay, P.; Crowley, M., and Bunting, R. W.: J. Dent. Research **12**:429, 1932; J. Am. Dent. A. **19**:265, 1932.

79. Rosebury, T., and Greenberg, S.: J. Dent. Research **12**:430, 1932.

Rats, finally, have organisms belonging to this group in their mouths constantly,[41b] probably because of fecal contamination, without developing caries; in rats with true caries the lactobacillus flora is not appreciably altered;[80] and the feeding of lactobacilli from human caries to rats on severe or mild caries-producing diets does not in either case increase the incidence of caries in their teeth.[25] In view of what has been said of the relation of diet to dental caries, although one may consider that dietary variation acts to establish, localize or inhibit the proliferation of lactobacilli in the mouth, it appears at present that factors other than infection are of determining importance in susceptibility to caries.

The relationship of the dental lactobacilli to L. acidophilus of Moro is of some importance to the field of intestinal "acidophilus milk" therapy. The dental organisms constitute a very heterogeneous group, morphologically, culturally and serologically; and whether they may properly be classed with L. acidophilus remains in dispute. McIntosh et al.[71] classed them as a variant, under the name B. acidophilus-odontolyticus. Morishita[81] compared organisms from teeth with the Moro bacillus and reported a number of differences; these have been cited by Rettger[82] as clear evidence of distinction. Morishita's results could not be confirmed by other workers,[83] however, who were unable to distinguish between the two groups. Beckwith[84] reported a difference in acid production, but I[85] was unable to confirm this. Pending final agreement on this point, I prefer the noncommittal term "lactobacilli" in reference to the dental organisms. The question is, it seems, an academic one; since, even though the dental lactobacilli should be shown to be both the effective agents in caries production, and identical with L. acidophilus, there is no evidence to indicate that feeding these organisms, as in intestinal therapy, need influence in any way the development or the course of dental caries.

The apparent importance of lactobacilli as agents in the production of dental caries brings up certain interesting questions concerning the mechanism of the process. The opinion is still occasionally encountered in the literature, for example, that many bacteria are potentially capable

80. Rosebury, T., and Greenberg, S.: Unpublished data.

81. Morishita, T.: Proc. Soc. Exper. Biol. & Med. **25**:654, 1928; J. Bact. **18**:181, 1929.

82. Rettger, L. F.: Yale J. Biol. & Med. **4**:485, 1932.

83. Rosebury, T.; Linton, R. W., and Buchbinder, L.: J. Bact. **18**:395, 1929. Howitt, B. F.: J. Infect. Dis. **46**:351, 1930. Hadley, F. P.; Bunting, R. W., and Delves, E. A.: J. Am. Dent. A. **17**:2041, 1930; **19**:28, 1932. Enright, Friesell and Trescher.[75b]

84. Beckwith, T. D.: Scient. Monthly **33**:267, 1931.

85. Rosebury, T.: J. Bact. **24**:321, 1932.

of decalcifying enamel (e. g., Hanke [47]). This objection to the hypothesis of specific action by lactobacilli has been answered in part by the finding [85] that lactobacilli possess notably greater acid-producing powers than other strong fermenters (Staphylococcus aureus and Bacterium coli); but the objection cannot yet be answered with finality. This question is bound up with the important subject of the solubility of dental enamel in acid solutions, which remains to be thoroughly worked out. Earlier studies had suggested that enamel does not dissolve at p_H levels above 4 or thereabouts, which would agree well with the concept of specificity, since only lactobacilli are able to reduce the p_H of artificial mediums below this level. But later studies have tended rather to indicate that no such critical level of enamel solubility exists. The work of Kanthak and Benedict [86] and Enright, Friesell and Trescher [75b] indicates that the solubility of enamel depends, not only on the p_H, but also on the salt (particularly calcium and phosphate) concentration of the solvent; and it is now known [87] that enamel may dissolve slightly in neutral or even alkaline solutions, although such solutions appear to remove quantitatively a constituent present in the tissue in small amounts. The recent work of Kanthak and Benedict attacked this problem from the point of view of the possible agency of the saliva in producing caries, and indicated that saliva contains a sufficient content of calcium and phosphate, even when the p_H is low, to prevent solution of enamel. It is probable, however, that the caries process, as it occurs in localized regions on the tooth surface, is protected from contact with saliva, whether through the agency of mucin plaques or otherwise.

A final point in connection with the bacteriology of dental caries relates to the possible agency of an immunologic factor in the problem of susceptibility. McIntosh et al.[71] found agglutinins to lactobacilli in the saliva of caries-free (?) persons in low dilution, but were unable to demonstrate any bactericidal activity. I [88] also investigated this subject and found only slight and inconsistent evidence of normal agglutinins to lactobacilli in the salivas of caries-free persons. The occurrence of lactobacilli in the mouths of persons without caries, and their uniform occurrence in the mouths of normal rats, make it doubtful that lack of susceptibility to caries can be determined by the presence of an inhibiting principle in saliva. Jay,[78b] however, recently revived interest in this matter by the report that agglutinins to lactobacilli are generally present in the serum in greater concentration in caries-free persons than in those with caries, and that susceptible persons generally show a cutaneous reaction on intradermal injection of filtrates of lactobacillus

86. Kanthak, F. F., and Benedict, H. C.: J. Dent. Research **11**:470, 1931.
87. Karshan, M.: J. Dent. Research **12**:523, 1932. Kanthak.[86]
88. Rosebury, T.: J. Dent. Research **10**:403, 1930.

cultures to a greater extent than those who are caries-free. The significance of these findings must, however, await further study.

SUMMARY

This paper reviews the clinical background of dental caries and the salient features of modern research into the problem. Dental caries, in its most characteristic and widespread form, is a disease of children, adolescents and young adults, presenting a definite clinical and histologic picture. It occurs characteristically at certain localized areas about the teeth which are food-retentive and difficult to keep clean, and proceeds from the surface of the enamel inward toward the pulp. Gram-positive, nonsporing bacilli belonging to the genus Lactobacillus are characteristically found in carious lesions. These organisms have unique powers of acid-production and acid-resistance, and since enamel is most readily destroyed by the action of acids, they probably bring about its destruction by their action on fermentable carbohydrate food particles at retentive sites. The saliva is probably never in itself an agent in the production of caries; it is potentially capable of neutralizing locally formed acid even when its p_H is low, by virtue of its buffer content, but the carious area may be protected against access by the saliva by mucin plaques or otherwise. Some factor concerned with the character of the diet is of importance in susceptibility to caries; the incidence of caries in children may be reduced by regulation of the diet, and typical caries may be produced in rats by dietary means; it now appears likely that this dietary factor is physical rather than chemical, acting locally in the mouth rather than through the agency of a dietary deficiency; but such deficiency may contribute to the process. The possible relationship to susceptibility to caries of other factors of a systemic or metabolic nature, and of factors influencing the structure of the teeth, cannot be excluded, but may be doubted pending the accumulation of additional evidence.

The reparative treatment of dental caries, based on the principle of local susceptibility, depending on dental conformation and natural hygiene, is invariably successful when properly practiced, in that dental caries does not recur under or at the margins of properly treated and filled cavities. Hygienic measures as now applied are probably of definite but limited value in combating caries. Empirical prophylaxis by regulation of the diet of children is distinctly effective, and, at least until the problem becomes more fully understood, deserves more universal adoption.

Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—Henry B. Ward, who retires from the headship of the department of zoology in the University of Illinois at the end of the current academic year, has been elected permanent secretary of the American Association for the Advancement of Science.

Guido Tizzoni, until recently professor of general pathology in the University of Bologna, and known especially for his pioneer work on tetanus and tetanus antitoxin, has died at the age of 79.

John Glaister, who held the chair of forensic medicine in the University of Glasgow from 1898 until 1931, when he was succeeded by his son, John Glaister, has died at the age of 76.

Cecil Fowler Beadles, curator of the pathologic department of the Royal College of Surgeons, London, has died at the age of 66.

Society News.—The next meeting of the German Pathological Society will be held on June 8 to 10, 1933, in Rostock. Georg B. Gruber, Planckstrasse 8, Göttingen, is the secretary.

W. Mansfield Clark is the newly elected president of the Society of American Bacteriologists; M. J. Rosenau is the vice president, and James M. Sherman, secretary-treasurer.

Nordhoff-Jung Cancer Prize.—The Sophie A. Nordhoff-Jung Cancer Prize for the best work in the field of cancer research in recent years has been awarded to Prof. Max Askanazy, of the University of Geneva, in recognition of his experimental work and his critical presentation of larger and general problems in the same field.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

EXPERIMENTAL EPITHELIAL METAPLASIA OF THE THYROID GLAND. T. YOSHIDA, Virchows Arch. f. path. Anat. **283**:29, 1932.

This report describes the changes that occurred in the thyroid glands of guinea-pigs following subcutaneous injection of o-amido-azotoluol, the chief constituent of Biebrich scarlet. From 1 to 2 cc. of a 1:10 to a 1:20 solution of the material in olive oil was injected at intervals of from one to three weeks. The material was toxic, causing the death of some animals and progressive loss of weight in those that lived. The animals died or were killed at intervals of from two to one hundred and fifty-four days after the first injection. In the experiments of longer duration, the thyroid gland was decreased in size. This decrease was due to degeneration and atrophy, associated with disappearance of colloid desquamation of epithelium and collapse of follicles. The degenerative changes were followed in six instances by regeneration of follicular epithelium. The latter was, however, of the multilayered squamous type and underwent hornification.

O. T. SCHULTZ.

EXPERIMENTAL RUPTURE OF THE ELASTIC TISSUE OF ARTERIES AND LIPOID INFILTRATION. A. SSOLOWJEW, Virchows Arch. f. path. Anat. **283**:213, 1932.

The carotid arteries of rabbits were isolated in tubular bridges of skin according to the technic of Cohn and Loewy. Cholesterol feeding was begun from 27 to 137 days after the operation. Five days after the institution of cholesterol feeding, at which time hyperlipoidemia was established, daily kneading and massage of the carotid arteries between the finger and thumb were begun. This procedure lasted from five to ten minutes and was repeated daily from 41 to 71 times. At the conclusion of the experiments, the inner surface of the vessels revealed grossly transverse yellowish ridges and striations such as are seen not infrequently in the human aorta. Microscopically, it was found that the manipulation to which the vessels had been subjected had resulted in rupture of the elastic tissue, especially that of the internal lamella. Healing occurred by proliferation of the tissue of the media and by new formation of elastic fibrils from the media. The endothelium of the intima did not take part in the process. The adventitia remained passive, unless rupture of the external elastic lamella had occurred, in which event adventitial fibrous tissue grew into the defect. Lipoid deposition occurred at the point of rupture of the elastic tissue, but only during the healing stages, and not after healing was completed. The "disposition" to lipoid infiltration is held to be due, in part, at least, to increased permeability of the wall of the vessel brought about by the break in continuity of the elastic fibrils.

O. T. SCHULTZ.

INFLUENCE OF CHANGES IN THE WEATHER ON APPROACHING DEATH. V. STRUPPLER, Virchows Arch. f. path. Anat. **283**:231, 1932.

At the München-Schwabing hospital, from which this article comes, the impression prevailed that the number of necropsies, and hence the number of deaths, increased decidedly following sudden and marked changes in the weather. This impression the author investigated by correlating the daily number of deaths, as they appeared in the necropsy record book, with the meteorologic conditions as recorded by the local weather bureau. The years 1928 and 1929 were selected for study. The daily average number of necropsies was 3, but there were many days with 10. In the graphic curve of daily deaths, days with 5 or more deaths presented definite peaks. Individual characteristics of the weather, such as tem-

perature, barometric pressure, etc., did not appear to be correlated with the death curve. But the meteorologic state, as expressed in combined factors such as barometric depression followed by a rise or a fall in temperature, humidity, change in air movement and storms, appeared to be correlated with increases in the daily number of deaths. Thus, 141 peaks in the necropsy curve were associated with such weather disturbances, whereas 14 peaks were not. When the deaths were grouped according to the main cause of death as determined at necropsy, changes in the weather appeared to have an influence on the number of deaths from tuberculosis; pneumonia, septic conditions of the lung and empyema; cerebral hemorrhage and softening, and cardiovascular disease. In all of these groups, a sudden change in the weather with rising temperature was followed by an increased number of deaths. A change with cold or falling temperature caused no apparent increase in the number of deaths from tuberculosis or from pneumonia and empyema. In tuberculosis, an increase in the number of deaths was correlated not only with disturbances of the weather characterized by rising temperature, but also, though to a lesser degree, with meteorologic states characterized by slight air movements. Deaths due to cardiovascular disease appeared to be increased by weather changes, both those accompanied by rising and those by falling temperature. Such effects as weather changes may have on approaching death, the author believes to be due to the state and movement of large air masses immediately above the surface of the earth of a given locality. Although other observers with larger necropsy material may be able to show that the author has not proved his point, his theme and his discussion of it are interesting.

O. T. SCHULTZ.

CHRONIC PURULENT POLYARTHRITIS. E. FREUND, Virchows Arch. f. path. Anat. **284**:374, 1932.

Purulent inflammation of one or more joints occurs usually as an acute and terminal process in fatal septic infection. Freund describes, as a distinct clinical and pathologic entity of rare occurrence, purulent inflammation that involved many joints and ran a prolonged clinical course. The two cases that he reports occurred in a man and a woman, each 68 years old. In the man, the disease had been present for five years, and in the woman, for twenty years. In each case, exacerbations with painful swelling of newly involved joints alternated with periods of fairly good general health during which use of the involved joints was possible. Grossly, the joint cavity was distended by thick fibrinopurulent exudate, the synovial membrane was thickened and its villi were hyperplastic and congested, and the cartilage was ulcerated in places. Microscopically, the cartilage, where it had not disappeared, was swollen, degenerated and in places necrotic. In joints that had been involved for longer periods of time healing occurred with fibrous ankylosis.

O. T. SCHULTZ.

PROSTATIC HYPERTROPHY, INTERSEXUALITY AND THE SEXUAL RELATIONSHIPS OF THE PROSTATE. L. MOSZKOWICZ, Virchows Arch. f. path. Anat. **284**:438, 1932.

The author's conception of prostatic hypertrophy and of the relation of the prostate to sex is based on a review of the literature of prostatic hypertrophy and on a discussion of the embryology of the prostate and of the development of this organ in pseudohermaphroditism. The normal prostate consists of two portions. The distal, which lies anterior to the colliculus seminalis, reacts to testicular hormone. The proximal, which lies posterior to the colliculus, is the part that undergoes hypertrophy in the later years of life. In the male embryo, the prostatic anlagen develop chiefly distal to the müllerian eminence, and in the female embryo, proximal to the eminence. In male, testis-bearing pseudohermaphrodites, the prostate lies distal to the opening of the vagina into the sinus urogenitalis; in female, ovary-bearing pseudohermaphrodites, it is situated proximal to the opening of the vagina into the sinus. The anterior part of the prostate is a male organ

the development of which is influenced by testicular hormone. It atrophies in later life, when the testicular hormone decreases or is lost. The posterior part is more closely related to female sex influences. Its hypertrophy in certain males indicates a state of intersexuality and a transformation toward femaleness.

O. T. SCHULTZ.

EXPERIMENTAL PRODUCTION OF AREAS OF SIDEROFIBROSIS IN THE SPLEEN. G. M. FASIANI and G. OSELLADORE, Virchows Arch. f. path. Anat. **284**:474, 1932.

The authors caused localized areas of necrosis in the spleen by the injection of a drop of alcohol or of concentrated calcium chloride solution or by electrocoagulation. To bring about stasis, the vessels supplying the injured areas were ligated at the hilus. The experiments were carried out on rabbits, cats and dogs. They yielded uniformly negative results in the rabbits, and uniformly positive results in the dogs, and in six of the twenty-eight cats. The lesions produced were identical with those seen in the human spleen. The fibrosis, with iron and calcium encrustation, was the result of focal degeneration and death of tissue, associated with stasis. The authors do not believe that stasis alone is sufficient to cause the lesions. The characteristic segmented, mycelium-like bodies, which have led some observers to conclude that the lesions seen in the human spleen are the result of infection with Aspergillus, were present in the experimental lesions. They were formed by the encrustation of elastic and collagen fibers.

O. T. SCHULTZ.

RELATION OF CHRONIC POLYARTHRITIS TO ACUTE RHEUMATIC INFECTION. F. KLINGE and N. GRZIMEK, Virchows Arch. f. path. Anat. **284**:646, 1932.

The sixth in a series of articles on rheumatic infection discusses the relation of chronic polyarthritis to this protean disease. The latter, termed febrile rheumatism by Klinge, has been pictured in previous communications as a disease that attacks the supporting tissues of the body widely. It may be divided into three clinical and anatomic types: the classic acute or subacute polyarthritis; the visceral type, attacking chiefly the heart, vascular system and serous membranes; the peripheral type, with involvement of the extremities. Common to the thirteen cases described in the present report was a gradual, afebrile onset, with involvement of multiple joints. The arthritis varied from chronic synovitis, that had been only a minor element in the clinical syndrome, to marked deforming arthritis of many large and small joints; in one case, the clinical diagnosis was gout. In most of the cases, the presence of characteristic granulomatous nodules, often of large size, in the periarticular tissues, of areas of fibrinoid degeneration in the synovial membrane or of lesions of the heart does not make it difficult to accept the view that these were cases of rheumatic infection. Two cases of marked deforming arthritis, with only pericardial thickening, scarlike areas of myocardial fibrosis and arteriosclerosis, make acceptance more difficult. Beginning in his first communication with the earliest stage of true febrile rheumatism, Klinge in this one broadens his conception of the disease to include cases of what others might term nonrheumatic chronic infectious arthritis, provided that careful examination reveals the presence of periarticular fibrous nodules or myocardial scars. These lesions the authors believe to be stigmas of rheumatic infection, even if such lesions are not histologically characteristic. The occurrence of secondary chronic polyarthritis in persons who have had rheumatic infection is not denied, but if the stigmas of rheumatism are present it is thought reasonable to assume that the chronic arthritis is an end-stage of true rheumatic infection. This end-stage may result in deforming arthritis or in fibrous ankylosis of joints. Klinge is an upholder of the anaphylactic theory of febrile rheumatism. The development of chronic polyarthritis, without the characteristic hyperergic stage, is discussed, as is also the relation of focal infection to chronic polyarthritis in the light of the view maintained.

O. T. SCHULTZ.

EFFECT OF LIGATION OF PANCREATIC DUCTS ON THE ISLANDS OF LANGERHANS. L. FALIN, Virchows Arch. f. path Anat. **284**:713, 1932.

Because of differences of opinion expressed by those who had investigated the effects of ligation of the pancreatic duct, Falin repeated their experiments, using roosters. This animal has a main duct that enters the duodenum separately from the bile duct, and from 1 to 3 accessory ducts. As many of the ducts as could be isolated were ligated. The animals were killed at intervals of from 71 to 240 days after operation. Animals in which all the ducts were ligated lost weight. If not all the ducts were ligated, the animal lost weight at first and then regained its normal weight. The blood sugar level and dextrose tolerance were not affected by the experimental procedure. The normal pancreas of the species used contain islet-like acini that are derived from larger acini. Similar pseudo-islets were formed in the atrophic process that followed ligation of the ducts. New formation of true islets and hypertrophy of preexisting islets were not observed. In the more extreme grades of acinar atrophy and fibrosis that followed ligation, some of the islets disappeared.

O. T. SCHULTZ.

REFLEX CONTROL OF THE CAPILLARY CIRCULATION OF THE LIVER. HEDWIG RACHOLD and G. RICKER, Virchows Arch. f. path. Anat. **284**:754, 1932.

In young rabbits, vessels belonging to the portal system, namely, the splenic or the superior mesenteric vessels, were subjected to a variety of procedures. The vessels were ligated or painted with mustard oil, or their adventitia was removed for distances of a few millimeters. The effect on the capillary circulation of the liver was studied by microscopic examination of the capillaries of the margin of the liver in the living animal and by examination of microscopic sections. The method of experimentation led to the opening up of new capillary paths connected with the preterminal and terminal capillaries of both the portal and the hepatic veins. These new vascular channels the authors term capillaments; they may become transformed into permanent capillaries. The action is believed to be a reflex one through centripetal fibers of the central nervous system running in the sheaths of the vessels of the portal system. The vascular reaction described occurs if the liver is anemic or if it is hyperemic as the result of digestion.

O. T. SCHULTZ.

EFFECT OF LIPOIDS ON THE SURVIVING HEART OF THE FROG. P. KIMMELSTIEL, Virchows Arch. f. path. Anat. **284**:835, 1932.

Previous attempts to determine the effect of lipoids on the heart are criticized by the author because impure preparations were used. In his investigation, the author used purified cephalin, lecithin and cholesterol, and a cerebroside mixture. As test object the surviving heart of a frog was used in its normal state, after fatigue, and after having been brought to a standstill by caffeine. The hydrophil lipoids cephalin and lecithin acted differently. Lecithin had a positive inotropic effect. Cephalin had a positive chronotropic effect and by its toxic action brought the heart to a standstill in diastole. The hydrophobe colloids cerebroside and cholesterol had little effect; they may protect the heart muscle by their property of adsorbing toxic substances. The hydrophil and hydrophobe colloids may have an antagonistic action. The effects of the lipoids may be due to their nonspecific physicochemical properties in some instances, and to their specific chemical properties in others. Attempts to determine the effects of lipoids by quantitative chemical analysis of human heart muscle must make use of methods that will quantitatively separate cephalin and lecithin.

O. T. SCHULTZ.

PHOSPHATID CONTENT OF THE BRAIN IN NIEMANN-PICK DISEASE. E. EPSTEIN, Virchows Arch. f. path. Anat. **284**:867, 1932.

The child with Niemann-Pick disease may exhibit symptomatic evidence of cerebral involvement, amounting in some cases to idiocy. Histologically, the brain reveals the same process of lipoid storage present in other organs. The lipoid material, which is chiefly the phosphatid lecithin, is present in ganglion cells, periadventitial cells and glia cells. A case reported by Pick was clinically one of typical familial infantile amaurotic idiocy with the characteristic retinal findings. The anatomic picture was that of typical Niemann-Pick disease. Pick suggested that the Tay-Sachs type of amaurotic idiocy may be a form of Niemann-Pick disease. Epstein examined chemically the brain of a child with Niemann-Pick disease and with the clinical symptoms of idiocy, but without the characteristic retinal changes of Tay-Sachs disease. Lecithin of the brain was increased 80 per cent above the lecithin content of the brain of a normal child of the same age; the lecithin content of the spleen was increased a hundredfold. The total phosphatid of the brain was increased 90 per cent; that of the spleen, nineteenfold. Epstein concludes that there are two types of Niemann-Pick disease, one with, the other without the retinal changes of infantile amaurotic idiocy. There is also a "pure" type of amaurotic idiocy, with retinal changes but without the characteristic alterations of Niemann-Pick disease in the liver, spleen and bone marrow.

O. T. SCHULTZ.

EFFECT OF EXPERIMENTAL SPLENECTOMY ON BLOOD PLATELETS AND BONE MARROW. D. GÖRÖG, Virchows Arch. f. path. Anat. **284**:917, 1932.

In three cases of essential thrombocytopenia, histologic examination of the spleen and bone marrow revealed no changes that would account for the low platelet count. In two cases of leukemia with symptomatic thrombocytopenia, replacement or damage of the megakaryocytes by the proliferated marrow tissue was evident. In an attempt to determine the relationship of the spleen to the number of platelets, rabbits were subjected to splenectomy. The operation was followed by a rapid increase in the number of platelets of the peripheral blood. The increase began on the first or second day after operation, reached its height of from three to four times the initial level on the fifth or sixth day, and then decreased more gradually, reaching the initial level between the fifteenth and the twentieth day. The increase in the number of platelets might be due to removal of a local platelet-destroying action of the spleen. The experiments yielded no evidence either for or against this possibility. The increase might be due to the removal of an inhibitory influence of the spleen on the bone marrow. There was no evidence that the spleen influences the rate of delivery of platelets from the marrow to the blood. At the height of the postoperative platelet increase, the megakaryocytes of the marrow were vacuolated. This alteration might be interpreted as evidence of partial exhaustion of previously overactive megakaryocytes. In all other respects the work yielded negative results.

O. T. SCHULTZ.

THE RELATION OF THE LIVER TO INFECTIONS OF THE BLOOD STREAM. T. NARITA, Ztschr. f. d. ges. exper. Med. **80**:303, 1932.

Healthy rabbits received intravenous injections of definite amounts of bacterial suspensions. Blood was removed, one hour later, from the jugular vein, and by cultural methods the number of organisms remaining in the blood stream was determined. The organisms used were a staphylococcus, a streptococcus, the colon bacillus, B. pyocyaneus, the Kartoffel bacillus and B. subtilis. The points of injection were the ear vein, the portal vein and the hepatic artery. In rabbits which received injections in the ear vein, after one hour the number of organisms

per cubic centimeter of blood was about one-half the original number, and this decrease occurred with each type of organism used. Two groups of animals were given injections simultaneously; in one the ear vein was used, and in the other the portal vein. After one hour the animals in which the ear vein had been used showed the greater number of organisms remaining in the blood. The difference was most marked with the Kartoffel bacillus and least marked with the colon bacillus. This same procedure, but with use of the hepatic artery instead of the portal vein, yielded similar though more striking results. In those animals, therefore, in which the bacteria had to pass through the liver before entering the general circulation, the bacterial count on the venous blood after one hour was less than in the group in which the bacteria had been injected by way of the ear vein. The defense mechanism of the liver appeared more active if the bacterial invasion was by way of the hepatic artery than if by way of the portal vein. When the reticulo-endothelial system was blocked with india ink emulsion, the ability of the animal to withstand an infection of the blood stream was diminished.

SANDER COHEN.

THE FUNCTION OF THE FETAL SPLEEN IN SPLENECTOMIZED PREGNANT RATS. A. BERGER and E. FLAUM, Ztschr. f. d. ges. exper. Med. **80**:531, 1932.

In splenectomized pregnant rats which have been infected with Bartonella there appears evidence of anemia with marked blood regeneration: Howell-Jolly bodies in the erythrocytes, megaloblasts, nucleated red blood cells and polychromasia. These changes appear from two to five days after splenectomy and do not occur in normal animals or in pregnant rats from which the spleens have not been removed. The conclusion is drawn that the fetal spleen is unable to take over the function of the maternal spleen in the splenectomized rat.

SANDER COHEN.

CHANGES IN THE GOLGI APPARATUS IN ALCOHOL POISONING. N. WASCHETKO and B. DEJKUN, Ztschr. f. d. ges. exper. Med. **81**:184, 1932.

In dogs which have received large doses of alcohol, by mouth, over varying periods of time, definite changes are found in the Golgi apparatus of the large pyramidal cells of the cerebral cortex. The nucleus and cytoplasm of the pyramidal cell are unchanged. In acute alcoholism, the Golgi apparatus is broken up into small granules scattered throughout the cytoplasm; in subacute and chronic intoxication with alcohol, it is present as small rodlike particles scattered diffusely through the cytoplasm or tending to be grouped in small perinuclear clumps. It is concluded that there is a definite relationship between the Golgi apparatus and the conditions necessary to maintain the life of the nerve cell.

SANDER COHEN.

THE RÔLE OF TEMPERATURE IN THE TREATMENT OF RELAPSING FEVER WITH ARSPHENAMINE. M. N. LEBEDEWA and N. W. GALANOWA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:298, 1932.

Mice infected with Borrelia duttoni were kept at 40 C. for two hours before and for a similar period after the injection of arsphenamine. Their brains were afterward found sterile, while those of more than half of the nonheated controls contained the infecting organisms. The result in mice suffering from a latent infection was equally successful. Experiments showed that it was not the heat alone that effected the sterilization, but the entrance of the chemical agent into the brain substance, as demonstrated by histochemical and quantitative methods. The quantity of arsenic in the brains of animals so treated was from five to ten times larger than that in animals given similar injections but not heated.

I. DAVIDSOHN.

TRANSMISSION OF CHICKEN LEUKOSIS TO CHICKS. J. ENGELBRETH-HOLM and A. ROTHE MEYER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:347, 1932.

Chicken erythroleukosis can be successfully transmitted to chicks a few days old. The disease shows in them more marked changes and a faster course than in the adult animals. The transmission was successful in all cases, even when injection of the same material into adult chickens did not produce the disease.

I. DAVIDSOHN.

DIFFERENCES IN DISTRIBUTION OF CHEMOTHERAPEUTIC AGENTS IN MICE DEPENDING ON THE SITE OF INJECTION. I. L. KRITSCHEWSKI and O. W. WINOGRADOWA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:410, 1932.

Arsphenamine was injected into the tail vein or into the left ventricle of the heart. The animal was killed immediately, and the distribution of the arsenic was studied by the histochemical method of Jancso. Very considerable differences were noted in the distribution of the compound, depending on the site of injection. In the lungs, when the injection was intravenous, the arsenic was found mainly in the capillaries of the alveolar walls, while with intracardiac injection it was absent in the capillaries but present in the large blood vessels. In the liver and spleen, following intravenous injection, the reticulo-endothelial cells were loaded with the drug, while following injection into the left ventricle it was present in a much smaller number of these cells. On the other hand, in the brain the endothelial cells of the capillaries were loaded with arsphenamine and the lumens of the vessels partially or completely occluded with thrombi containing this compound only after intracardiac injection, while very little, if any, arsenic was found in them after intravenous injection. The same differences in distribution were seen when small therapeutic doses were injected.

I. DAVIDSOHN.

Pathologic Anatomy

POST-VACCINIAL ENCEPHALITIS. A. H. GORDON and L. J. RHEA, Am. J. M. Sc. **184**:104, 1932.

In a case of postvaccinal encephalomyelitis, the spinal cord showed the following changes: areas of demyelinization, extending for some distance around the vessels and unassociated with vascular thrombosis; infiltration of the meninges with lymphocytes, plasma cells and monocytes; perivascular lymphocytic infiltration. Gordon and Rhea consider the demyelinization to be the primary lesion and the cellular reaction secondary.

SANDER COHEN.

MICROSCOPIC CEREBRAL HEMORRHAGE IN STILLBIRTHS AND NEWBORN DEATHS. F. A. HEMSATH and M. M. CANAVAN, Am. J. Obst. & Gynec. **23**:471, 1932.

Microscopic examination of a single or of two sections of each of an unselected series of fifty-three medullae oblongatae from new-born infants who died and from stillborn infants revealed microscopic hemorrhages in thirty-four (64 per cent). In twelve, this finding supplied an anatomic explanation of death, which would otherwise have been lacking.

AUTHORS' SUMMARY.

OSTEITIS FIBROSA. F. J. LANG, Am. J. Path. **8**:263, 1932.

Von Recklinghausen advanced the opinion that, in the bony system, the secondary symptoms and sequelae control the disease picture and mask the basic factors. This important conception explains the great difficulty in arriving at a definite conclusion from the final picture of the disease process. It has been shown

that in rickets, scurvy and osteomalacia, the disease picture is dominated by osteitis fibrosa. It is only by exacting histologic methods, designed to show both the calcified and the noncalcified bone, and by the study of many large sections from various bones, seeking the primary changes, unobscured by osteitis fibrosa, that one is able to establish the basic nature of the disease. Without such careful methods, an incorrect diagnosis of primary osteitis fibrosa will be made, thus confirming the current misconceptions on this subject. Of all the conditions that are responsible for the origin of osteitis fibrosa, the most decisive are the peculiar structure and the circulatory system of bone which allow only slight compensatory adapation to circulatory disturbances. Furthermore, under the given pathologic conditions, use of the bone acts as an irritant. The functional point of view has proved to be of the greatest importance in explaining the question of the relation between osteitis fibrosa, osteomalacia and rickets. Likewise, in general pathology, the application of the functional point of view promises to clarify the origin and relationship of many obscure changes.

AUTHOR'S SUMMARY.

SUBCUTANEOUS NODULES IN CHRONIC ARTHRITIS. B. J. CLAWSON and M. WETHERBY, Am. J. Path. **8**:283, 1932.

Two hundred patients who had chronic arthritis were studied to determine the frequency of subcutaneous nodules in this disease. Nodules varying in diameter from 5 mm. to 3 cm., and in number from one to several per patient, were found in 29.5 per cent of the cases. Structurally, these nodules seemed to be similar to those found in acute rheumatic fever and to those produced in animals by injecting streptococci. They tended to be larger in chronic arthritis. The microscopic structure was chiefly polyblastic, but small areas of necrosis and abscess formation were commonly seen. When these nodules were macerated under sterile conditions and cultured in beef infusion dextrose broth, diplococci morphologically and culturally similar to those commonly found in the blood of patients having acute rheumatic fever or chronic arthritis were isolated in 70.6 per cent of the cases. The frequency of the subcutaneous nodules in acute rheumatic fever and chronic arthritis, the similarity of the gross and microscopic structure of the nodules in these two conditions and in experimental streptococcic nodules, and the frequency with which streptococci can be cultured from the blood in acute rheumatic fever and from the blood and the nodules in chronic arthritis strongly suggest that acute rheumatic fever and chronic arthritis for the most part have a common streptococcic etiology, and that the two diseases are in all probability different manifestations of the same process.

AUTHORS' SUMMARY.

EFFECT OF RADIUM EMANATION ON THE HISTOCYTE IN THE LIVER OF THE WHITE RAT. G. M. HIGGINS and J. C. T. ROGERS, Am. J. Path. **8**:355, 1932.

A study has been made of the reaction of the histiocytes in the liver to radon, as contained in gold radon seeds. Similar but inactive gold seeds were used in a series of control animals to determine the local reaction to such foreign bodies. All local histiocytes within the vicinity of the inactive gold seed responded immediately to the foreign body by retracting their processes and either desquamating into the sinusoids or migrating into adjacent parenchyma. Their function as littoral histiocytes had apparently ceased, and they were now concerned in an attack on the foreign body. Seventy-two hours after insertion of the inactive seed into the parenchyma, an effective barrier had been formed around it by these actively migrating histiocytes. Subsequently these cells contributed toward the formation of connective tissue. All histiocytes in the vicinity of the active radon seed were as susceptible to the emanation as were the cells of the parenchyma. The extent of the destruction by the radon increased until the maximal injury was reached at about the thirty-fifth day. Histiocytes within the zone of injury were destroyed, and those immediately beyond did not manifest signs of restorative activity during this period. After the fifth week, however, a retarded restorative activity was

manifested by the histiocytes just peripheral to the zone of maximal injury. By the tenth or twelfth week those cells which were considerably remote from the radon seed, and which either had not been affected or were only slightly affected by the radon, migrated rapidly toward the seed and contributed toward the formation of a wall comparable to that seen three days after the implantation of the control seed.

AUTHORS' SUMMARY.

THE ORIGIN OF EPITHELIUM-LINED BLOOD CYSTS (CHOCOLATE CYSTS) FROM THE GRAAFIAN FOLLICLE AND ITS DERIVATIVES. E. S. J. KING, Am. J. Path. **8**:417, 1932.

In the course of its development, the graafian follicle give rise to a varied series of structures, the advanced stages of which bear little morphologic resemblance to the parent structure. A study of the intermediate stages is necessary for the demonstration of the relationship of the atypical forms to the original follicle. Hemorrhage into these structures occurs so frequently as to be almost a normal phenomenon. A heterotopic epithelial lining develops into the older cysts which contain blood. Since these older cysts, as stated, bear but little resemblance to the original follicles, the relationship has been overlooked. These cysts are identical with those which, on account of the nature of the epithelium, have been described as "endometrial."

AUTHOR'S SUMMARY.

TRANSIENT THICKENING OF THE AORTIC INTIMA IN CHILDHOOD. C. M. TORRES, Am. J. Path. **8**:455, 1932.

In childhood, the aortic intima may vary in thickness, corresponding to transient and recurrent developmental changes. Quite marked thickenings of the intima may disappear soon after they are formed as the growth of the aorta proceeds. "Developmental changes associated with degenerative processes are a pitfall in the diagnosis of juvenile arteriosclerosis in infancy. In some children, fatty changes were seen in the absence of developmental thickenings of the intima, and in others, on the contrary, quite marked simple developmental changes were found in the absence of degenerative processes."

PNEUMONIA FROM ASPIRATION OF FAT AND OIL IN THE LUNGS. J. W. PIERSON, Am. J. Roentgenol. **27**:572, 1932.

Fats and oils administered to children either orally or through the nasopharynx often reach the alveoli of the lungs. They collect in large masses in the lungs and are associated with chronic pneumonia and fibrosis. The widespread use of oils in pediatrics offers a large potential source for this condition. The presence of fatty material in the lungs produces an interesting roentgen picture, but no pathognomonic signs are offered. This communication is presented with the hope that it may stimulate roentgenologists to make such observations as will aid in the diagnosis of this condition.

AUTHOR'S SUMMARY.

OSTEOPOIKILOSIS: DISSEMINATED CONDENSATION OF THE BONES. L. F. WILCOX, Am. J. Roentgenol. **27**:580, 1932.

Osteopoikilosis is probably not so rare as believed. I have attempted to tabulate all the authentic cases reported in medical literature. In the two cases that I have reported, in father and son, the similarity of the skeletal disturbances proves, in my estimation, that the condition is hereditary, as was suggested by Voorhoeve. In Schmorl's case which was studied at autopsy, no mention was made of abnormality in the parathyroid glands. It is of interest to speculate that osteopoikilosis may be associated with disturbance of these glands. It is hoped that if further occasion permits study of this condition at autopsy, the parathyroid glands will be investigated.

AUTHOR'S SUMMARY.

PECULIAR FORM OF NEPHRITIS ASSOCIATED WITH ACQUIRED SYPHILIS. A. R. RICH, Bull. Johns Hopkins Hosp. **50**:357, 1932.

A hitherto unrecognized renal lesion associated with syphilis is described and illustrated. The renal cortex presents glistening, grayish-yellow spots, which on the cut surface appear as gray or grayish-yellow streaks in which are shining flecks. There may be scars in the surface surrounded by sandlike glistening points. The lesions consist of dense foci of small lymphocytes chiefly, but other mononuclear cells are present. These foci tend to form spherical masses, which encroach on the tubules and project into the lumens. This is a striking characteristic. In the lumen of the affected tubule may be cells of various kinds and cellular débris, in which are the cholesterol crystals to which are due the glistening points seen macroscopically. This unique lesion has been found in 6.5 per cent of two hundred autopsies in which the other organs contained syphilitic lesions; also in cases without syphilitic lesions of the organs, but with clinical evidence of syphilis; but not in four hundred autopsies in which there was no indication of syphilis. So far the spirochete has not been found in the lesion.

MUCUS SECRETION IN THE TRACHEA. H. FLOREY, H. M. CARLETON and A. Q. WELLS, Brit. J. Exper. Path. **13**:269, 1932.

The immediate effects of various types of irritants are described. Chronic irritation of mucous membranes such as those of the trachea, bronchi and small intestine leads to a great increase in the number of goblet cells. In the trachea, more intense irritation causes the columnar and goblet cells to be replaced by a stratified epithelium rather similar to that of the urinary bladder. Gland cells that on a morphologic basis would be termed "serous" appear often to be capable of elaborating mucus, as evidenced by their histochemical reactions.

FROM AUTHORS' SUMMARY.

SPECIFICITY OF TISSUE AND BLOOD REACTION. W. F. HARVEY and T. D. HAMILTON, Edinburgh M. J. **39**:349, 1932.

There is an invariable sequence of events in response to the introduction of foreign material into the body, a polymorphonuclear infiltration followed by a monocytic and, if time allows, a lymphocytic. The lymphocyte, monocyte, macrophage and giant cell probably represent stages of development of one and the same type of cell. No matter what the nature of the stimuli—trauma; injection of salt solution, india ink, staphylococci, tubercle bacilli or benzene; ingestion of food or fluid; posture; application of heat; muscular exercise—they all produce the same sequence of events so far as cellular response in the tissues or in the blood is concerned. There is not yet sufficiently conclusive evidence, except by extension of the local influence and of deficiency of cells, for chemical action at a distance as a factor in the production of a cellular response of blood and tissue. Vasomotor and sympathetic nerve stimulations, which produce distant effects, are of the nature of propagated nerve stimuli. One may speak of a positive chemotaxis in the sense of collection of cells to a focus, but not in the sense of a specific call for a special type of cell. It is doubtful whether negative chemotaxis exists. The special tissue manifestations and blood pictures attached to particular diseases and assisting in their diagnosis are reducible to manifestations and simple acuteness or chronicity, local tissue destruction, and duration of stimulus, thus making the blood picture, especially, a matter merely of the time at which the examination is made. There is nothing specific about the cellular reaction in disease or in physiologic states. There is only variation in the degree and duration of a phase.

AUTHORS' SUMMARY.

ANATOMICAL CAUSES OF HEART BLOCK. R. T. and P. D. GRANT, Heart **12**:142 and 145, 1932.

In one case, complete heart block was caused by the destruction of the terminal part and the bifurcation of the auriculoventricular bundle by an arterial angioma. In another case, in which the ventricular complexes varied in form, the bifurcation of the auriculoventricular bundle was destroyed by a calcareous mass, the main bundle being separated from its branches and the right branch from the left.

MORPHOLOGY OF THE BILE DUCTS IN MECHANICAL ICTERUS. R. RABL, Beitr. z. path. Anat. u. z. allg. Path. **86**:135, 1931.

From a study of the liver in spontaneous (human) and experimentally produced (dog) obstruction of the common bile duct Rabl concludes: Obstruction of the common bile duct produces hyperemia and loosening of the Glisson framework of the liver with separation of the cells. This permits communication of the intercellular biliary clefts with the spaces of the supporting framework and secondarily with the perivascular lymphatics. Necrosis of cell complexes is not a prerequisite, and only later does direct communication of the liver clefts and the perivascular lymphatics occur, when more marked separation of the cells is permitted by the markedly loosened Glisson framework.

W. S. BOIKAN.

HYALINE EXCRETION DROPLETS IN THE KIDNEY. A. TERBRÜGGEN, Beitr. z. path. Anat. u. z. allg. Path. **86**:235, 1931.

Contrary to previous belief, the hyaline droplets found in the renal tubules under certain conditions are not degeneration products, but excretion products, the histologic evidence of active cellular excretion of foreign proteins. In healthy persons killed suddenly, such droplets are not found. The latter are always found in association with conditions in which there occurs a breaking down of tissue—as after operations, injuries, burns, amyloidosis, injections of serum and cancer and in inflammatory diseases. In uremia, the droplets are seldom seen; in glomerulonephritis with destruction of tissue in other parts of the body, more often. In some cases, their occurrence depends on vascular injury and increased permeability of cell membranes, as in experimental temporary arterial ligation. But in all cases their presence is a sign of normal function, not of degeneration.

W. S. BOIKAN.

FUSION OF THE CHORDAE TENDINEAE AND PAPILLARY MUSCLES. D. WYROPAEW, Centralbl. f. allg. Path. u. path. Anat. **54**:86, 1932.

The author studied fusion of the papillary muscles and chordae tendineae in the heart of a 25 year old man who died of mitral endocarditis. The fusion was due to a rheumatic Aschoff granuloma between the muscles, with no involvement of their deeper portions.

GEORGE RUKSTINAT.

XANTHOMATOUS TRANSFORMATION OF THE MESENTERY. E. PETRI, Centralbl. f. allg. Path. u. path. Anat. **54**:113, 1932.

A marked lipoid infiltration was found in the mesentery of a 69 year old diabetic woman who died of cancer of the vulva. The diabetes was of such severity that the blood sugar was 0.26 Gm. per hundred cubic centimeters, and about 60 Gm. of sugar appeared in the urine daily. At autopsy, the mesentery was 7 cm. thick and occupied most of the abdominal cavity. Chemical analyses revealed that 7.18 per cent of the fat in the mesentery was cholesterol, about ten times the usual amount. Microscopically, xanthoma cells were demonstrable as were also cholesterol crystals. Cholesterol was proved present polariscopically also.

GEORGE RUKSTINAT.

THICKENING OF PULMONARY APEX AND SPLENIC CAPSULE. L. LOEFFLER, Centrabl. f. allg. Path. u. path. Anat. **54**:118, 1932.

Loeffler noted the frequent occurrence of thickening of the pulmonary apex and splenic capsule in the bodies of people over 50 years old. The condition in the lung is often found in people under 50. He believes the mechanical bumping action of the diaphragm may be responsible for both conditions.

GEORGE RUKSTINAT.

BORDERLINE CASES OF PERIARTERITIS NODOSA. H. KLINGER, Frankfurt. Ztschr. f. Path. **42**: 455, 1932.

Two instances of periarteritis nodosa which clinically simulated cases of sepsis are described. In both, typical changes in the spleen and lungs were found. In the first instance, these organs and the upper respiratory tract showed the only localizations of the disease; in the second instance, the kidneys and testes were also involved. In both cases, subacute and subchronic glomerulonephritis was encountered. In the first instance, there was marked inflammation of the adventitial regions, combined with subintimal inflammation. The inflammatory exudate was mainly purulent, but granulation tissue was present. In the second instance, necrosis was in the foreground. There was no evidence of syphilis in either case, histologically or according to the history. The author believes that a primary functional change in the blood vessel in the sense of Ricker is not likely to be the underlying etiologic factor.

O. SAPHIR.

HISTOLOGIC CHARACTERISTICS OF BASAL CEREBRAL ARTERIES. J. VONCKEN, Frankfurt. Ztschr. f. Path. **42**:481, 1932.

There are defects in the media at the point of division of the cerebral basal arteries. These defects are found either with or without compensatory hypertrophy of other layers of the arterial wall. They may be the basis of aneurysmal formations. Their location and distribution correspond closely to the location and distribution of aneurysms. They may also contribute to changes in the brain and meninges.

O. SAPHIR.

DIFFUSE DEMYELINIZATION OF THE BRAIN (SCHILDER'S DISEASE). G. PATRASSI, Virchows Arch. f. path. Anat. **281**:98, 1931.

The disease described by Schilder in 1912 as encephalitis periaxialis diffusa has been encountered chiefly in children. Patrassi's patient was a boy, aged 10. The onset of the disease was a sudden attack of vertigo, without vomiting or loss of consciousness, while he was in school. Apparent recovery after two days was followed by eight or nine repeated attacks, between which the child appeared well. Difficulty in vision set in, and bilateral papilledema was noted. Neurologic manifestations, other than dilatation and sluggishness of the pupils, were indefinite. At necropsy there were found in the white matter of both cerebral hemispheres areas of gelatinous degeneration. The histologic changes noted in the involved areas were: degeneration of the myelin sheaths and destruction of the bared axis cylinders; proliferation of macroglia and formation of fibrillated astrocytes; proliferation of microglia and oligodendroglia, with the formation of phagocytic granule cells that contained droplets and granules of lipoid material; moderate perivascular lymphocytic infiltration. From his study of reported cases, Patrassi believes a constitutional vulnerability of the white matter of the brain to be the most important underlying factor. This constitutional factor may be hereditary, familial or individual. In many cases, but not in all, a toxic-infectious factor is evident. He discusses the comparative histology of multiple sclerosis and diffuse sclerosis. The latter he believes to be a late stage of the disease described by Schilder. He would discard the name of encephalitis periaxalis diffusa, because the inflammatory nature of the process has not been established, and would substitute therefor the name leuko-encephalopathia myeloclastica primitiva.

O. T. SCHULTZ.

NONPURULENT POLYMYOSITIS. E. VON ZALKA, Virchows Arch. f. path. Anat. **281**:114, 1931.

The reported case of polymyositis occurred in a man aged 36, who had acute gonorrhea and a history of having had syphilis. The disease ran a septic course and terminated fatally in twenty days. In the various voluntary muscles there were focal areas of degeneration and necrosis of the muscle fibers, without leukocytic infiltration. Bacterial organisms and spirochetes could not be detected in the lesions by appropriate staining methods. The cardiac musculature was also involved. The author ascribes the condition to a rhabdomyotropic toxin or virus of unknown origin and nature.

O. T. SCHULTZ.

HISTOCHEMICAL DIFFERENTIATION OF DISEASES CHARACTERIZED BY LIPOID INFILTRATION OF CELLS. E. EPSTEIN, Virchows Arch. f. path. Anat. **281**: 152, 1931.

This report is based on the study of material from three types of lipoid infiltration, or lipoidosis. The three types are usually designated Niemann-Pick disease, Gaucher's disease and Schüller-Christian disease. The first is characterized by the presence within the cells of the phosphatide, lecithin; the second, by the cerebroside, kerasin, and the third, by cholesterol and cholesterol esters. A terminology based on these physicochemical differences would be preferable to the present eponymic nomenclature. The cholesterol and cerebroside lipoidoses result from the vital storage of the contained material by histiocytes; phosphatide lipoidosis is the result of fatty degeneration.

O. T. SCHULTZ.

TRANSFORMATIONAL POTENCIES OF THE LYMPHOCYTE. RUTH KATZENSTEIN, Virchows Arch. f. path. Anat. **281**:172, 1931.

The findings are based on study of aseptic inflammation of the abdominal wall of the rabbit and of tissue cultures of the white blood cells of the same species. Most of the cells of the inflammatory exudate are derived from the blood stream. The emigrated granulocytes degenerate and die. The lymphocytes, which leave the blood stream as do the granulocytes, are transformed into polyblasts, resting wandering cells and fibrocytes. The monocytoid lymphoid cell is not a distinct cell form, but is an ordinary lymphocyte in process of transformation to a polyblast. Monocytes are not the sole source of polyblasts, which come from resting wandering cells as well as from lymphocytes. Resting wandering cells and fibrocytes are not transformed into blood cells. The undifferentiated mesenchymal cells of the tissue take part in the inflammatory reaction and are transformed into histiocytes, fibrocytes and hemocytoblasts.

O. T. SCHULTZ.

SPONTANEOUS SUBCAPSULAR RUPTURE OF THE SPLEEN. E. HACKER, Virchows Arch. f. path. Anat. **281**:191, 1931.

In a woman, aged 36, who died of acute vegetative endocarditis of the tricuspid valve of five weeks' duration, there was found a subcapsular hematoma of the spleen, due to spontaneous rupture of the pulp.

O. T. SCHULTZ.

CHANGES IN THE VERTEBRAL COLUMN IN ACROMEGALY. J. ERDHEIM, Virchows Arch. f. path. Anat. **281**:197, 1931.

This hundred-page dissertation is dedicated by Erdheim to Gustav Pommer in honor of the latter's eightieth birthday. Erdheim speaks of it as a continuation of his studies previously published in a monograph devoted to the changes in the costal cartilages and joints in acromegaly. The alterations noted in the acromegalic vertebral column have hitherto been considered identical with those of spondylitis deformans. With this view Erdheim does not agree. The vertebral

column that formed the basis of the present study came from a woman aged 48, whose acromegalic abnormalities of growth had been evident since the age of 28. In order that specific changes, if present, might be properly evaluated, the vertebral column of a nonacromegalic person of the same age and sex was subjected to equally painstaking and thorough microscopic study. The description of the changes seen in the normal spine and their comparison with those of the acromegalic spine constitute the first half of Erdheim's contribution.

The most characteristic change in acromegaly is an increase in the transverse and anteroposterior diameters of both the vertebral body and the intervertebral disk. This alteration, which can be detected roentgenologically, has in the past been interpreted as a decrease in the vertical measurement of the vertebrae. The thickness of the body and disk is not actually decreased, but only appears so because of the actual increase in the other measurements. The increase is due to marginal subperiosteal new formation of bone in the vertebral body and to marginal subperichondrial new formation of cartilage in the disk. These processes imitate closely the growth of the disk and vertebral body in the early years of life. The growth process has nothing in common with that of spondylitis deformans. It is identical with the overgrowth that occurs in other parts of the skeleton in acromegaly, and is a specific result of the hypopituitary state. Other changes noted, especially in the disk, are idential with those of the normal spine and are the result of advancing age and mechanical factors. The most important of these alterations are albuminoid granular degeneration of the ground substance and fibrils of the nucleus pulposus, mucoid degeneration of the ground substance and fibrils of the annulus fibrosus, and asbestos-like degeneration of the ground substance of the cartilaginous plate. These degenerative changes may lead to the formation of small clefts and spaces. Microfractures of the zone of preliminary calcification and of the adjacent substantia spongiosa of the body were noted in both the normal and the acromegalic spine. The degenerative and traumatic alterations are followed by reparative proliferation; in the acromegalic spine, the reparative proliferation in response to nonspecific alterations was much more pronounced than in the control normal spine. The article is difficult for the reader of English, because of its wealth of histologic detail and rather complicated style. Also he will be disappointed by the apparent absence of a summarizing statement. However, he will find that subdivision 8 of main division B, on pages 279 to 286 inclusive, is a fairly succinct statement of Erdheim's findings and conclusions.

O. T. SCHULTZ.

ANATOMY AND PATHOLOGY OF THE LARYNGEAL VENTRICLE. H. SCHRÖDER, Virchows Arch. f. path. Anat. **281**:330, 1931.

In one hundred fresh necropsy specimens, the size, length and contour of Morgagni's pouch of the larynx bore no relation to age or sex. Lymphoid tissue, although present in the wall, is not relatively prominent enough to warrant considering the pouch a lympho-epithelial organ. Enlargement of the reaction centers of the lymphoid follicles was frequently seen, but no relation between hyperplasia of the lymphoid tissue of the pouch and status lymphaticus was noted. Characteristic pathologic changes were seen only in tuberculosis and diphtheria. The multiplication of diphtheria bacilli in the pouch may lead to the carrier state.

O. T. SCHULTZ.

Microbiology and Parasitology

GROWTH REQUIREMENT OF STAPHYLOCOCCI. T. P. HUGHES, J. Bact. **23**:437, 1932.

The substance present in meat extract capable of stimulating the growth of staphylococci belongs to the chemical group of "natural bases." It is heat stable in slightly acid or neutral solutions, but rapidly destroyed in alkaline solution. It

dialyzes through collodion membranes. This activating substance is soluble in water, alcohol and acetone, but insoluble in ether, ethyl acetate or benzene. It is precipitated by phosphotungstic acid and silver barium, but not by silver nitrate, mercuric chloride, acidic solution of mercuric sulphate, picric or picrolonic acids. It is apparently strongly adsorbed on metallic sulphides. It can be concentrated to a degree at which less than 1 mg. is sufficient to activate 50 liters of synthetic medium, thus permitting good growth of staphylococci. In chemical and growth-promoting properties, it behaves similarly to a substance produced intracellularly by B. coli-communior, an organism that grows profusely in synthetic mediums. Such a substance is not produced by staphylococci, which do not grow on synthetic mediums.

AUTHOR'S SUMMARY.

TUBERCLE BACILLI IN THE BLOOD STREAM OF RABBITS DURING THE COURSE OF INFECTION. L. MISHULOW and W. H. PARK, J. Prev. Med. **6**:95, 1932.

From our two experiments it seems probable that there is rapid localization of the tubercle bacilli inoculated into the blood stream, as shown by the tremendous decrease in their number between twelve and twenty-four hours after inoculation, and the steady decrease up to the fourth day. The organisms persisted in the blood stream throughout the entire course of the infection, although they fluctuated in number from day to day. There was a marked rise in number on the day of death. This would justify the conclusion that there is a steady dissemination of the tubercle bacilli from the local lesion into the blood stream.

AUTHORS' SUMMARY.

THE EFFECT OF BRILLIANT GREEN ON EBERTHELLA DYSENTERIAE, SONNE TYPE. E. M. MYERS and S. A. KOSER, J. Prev. Med. **6**:101, 1932.

Development of the Sonne type of E. dysenteriae was found to be markedly restrained by the addition of brilliant green to broth, to 2 per cent peptone water and to agar in dilutions that permitted development of E. typhi and members of the Salmonella group. Cultures of the Sonne type appeared to be as sensitive to the effect of the dye as Bacterium coli. For this reason, graded dilutions of the dye could not be used for enrichment of the Sonne type with simultaneous inhibition of organisms of the colon group. Several cultures of E. dysenteriae Flexner and of E. dispar were also inhibited by brillant green in as high dilutions as were the Sonne cultures. In brilliant green-bile mixtures the inhibitory effect of the dye on the Sonne type was also apparent. The use of brilliant green-bile for enrichment and subsequent isolation of this organism from experimentally inoculated samples of feces appeared to offer no advantage over direct plating of the specimen. Eosin methylene blue (methylthionine chloride U. S. P.) agar apparently exerted little or no restraining effect, and the Sonne cultures developed readily on this medium.

AUTHORS' SUMMARY.

TYPHUS FEVER: EXPERIMENTAL TRANSMISSION OF ENDEMIC TYPHUS FEVER OF THE UNITED STATES BY THE RAT FLEA (XENOPSYLLA CHEOPIS). R. E. DYER, E. T. CEDER, R. D. LILLIE, A. RUMREICH and L. F. BADGER, Pub. Health Rep. **46**:2481, 1931.

In conclusion it may be stated that the rat flea (Xenopsylla cheopis) as a vector of endemic typhus meets the requirements of the epidemiologic evidence. The virus of endemic typhus has been recovered repeatedly (four times by us; once by Kemp) from rat fleas taken at foci of typhus, and, finally, experimental transmission of the virus from rat to rat by means of the rat flea (X. cheopis) has been carried out in the laboratory. The foregoing evidence points to the rat flea (X. cheopis) as a common vector of endemic typhus from rat to rat and from rat to man.

AUTHORS' SUMMARY

THE "KILLING" OF BACTERIA BY BACTERIOPHAGE. C. H. ANDREWES and W. J. ELFORD, Brit. J. Exper. Path. **13**:13, 1932.

A specific phage added in excess to colon bacilli can within a few minutes, perhaps within a few seconds, so affect the organisms that they are rendered incapable of propagation; in other words, it apparently kills them immediately. At 37 C., lysis of these killed organisms follows in from twenty to thirty minutes. At 20 C., though the killing has taken place at once, lysis may not be demonstrable or may occur only after some hours. Sodium citrate seems to prevent multiplication of this colon bacillus phage. It cannot prevent killing of colon bacilli if phage is added in excess, but such killed phage will not lyse if citrate is present. The experiments described, with a strain of B. coli and a homologous phage, will not necessarily be reproducible with any other race of bacteriophage.

AUTHORS' SUMMARY.

MICROCHEMICAL STUDIES OF NONACID-FAST TUBERCLE BACILLI. H. MÖLLGAARD, Beitr. z. Klin. d. Tuberk. **79**:515, 1932.

It was possible, by a new staining method which is described in detail, to demonstrate in tuberculous foci and in blood cultures from tuberculous patients the presence of nonacid-fast bacterial forms. They seem to be identical in morphology and tinctorial characteristics with the spores and the hyphae of an actinomyces which the author has described before. The presence of these organisms in all inflammatory tuberculous lesions suggests its pathogenetic relation to tuberculosis. Pure cultures of this organism produce tuberculosis in guinea-pigs with the appearance of typical acid-fast bacilli. It is believed, without definite proof as yet, that the sporelike organisms are identical with the so-called filtrable phase of the tubercle bacillus.

MAX PINNER.

ISOLATION OF TUBERCLE BACILLI FROM PLEURAL EFFUSIONS. N. LEVIN, Beitr. z. Klin. d. Tuberk. **79**:616, 1932.

Ninety-one pleural effusions were examined for tubercle bacilli by cultural methods; 72 per cent of them contained bacilli. The lowest percentage of positive results was obtained in serous pleuritis.

MAX PINNER.

INCREASE IN VIRULENCE OF THE CALMETTE BACILLUS. H. MUCH and A. HAIM, Beitr. z. Klin. d. Tuberk. **79**:665, 1932.

Virulence tests were done on two strains which were received from Lübeck and which were allegedly B C G. In various methods of application it was found that both strains are slightly virulent, particularly when injected with tuberculin or with lactic acid. Although the exact history of the strains (possible contamination with a virulent human strain) is not known, it is concluded that the experiments prove the possibility of increasing the virulence of B C G under certain conditions.

MAX PINNER.

THE MORPHOLOGIC VARIABILITY OF THE TUBERCLE BACILLUS (HUMAN TYPE) IN CULTURES. P. KALLÓS, Beitr. z. Klin. d. Tuberk. **79**:688, 1932.

Tubercle bacilli were suspended in sterile olive oil and were then floated on broth. Growth occurred both on the surface and in the depths of the fluid. In the surface growth atypical bacilli were found, such as very long ones, free gram-negative granules and organisms with true branching.

MAX PINNER.

THE ETIOLOGIC DIAGNOSIS OF PULMONARY TUBERCULOSIS IN CHILDHOOD. G. W. SCHMIDT, Beitr. z. Klin. d. Tuberk. **79**:713, 1932.

In order to ascertain the presence of tubercle bacilli in the largest possible number of cases of pulmonary tuberculosis in childhood, it is necessary to examine the sputum, gastric contents and feces. All microscopically negative specimens should be injected into guinea-pigs, since the demonstration by inoculation is superior to the demonstration by pure culture.

MAX PINNER.

THE PRIMARY INTESTINAL TUBERCULOUS COMPLEX. M. IIZUKA, Beitr. z. Klin. d. Tuberk. **80**:79, 1932.

The study is based on the examination of 51 patients with an intestinal primary focus. Their ages varied from 20 to 87 years. The portal of entry is usually the lower ileum of the cecum. The intestinal lesion heals completely and is usually not demonstrable. The lymphoglandular component is usually demonstrable in multiple mesenteric lymph nodes. It resembles in all details the lymphoglandular component of a primary pulmonary focus. Deposits of lime salt are usually pronounced, and metaplastic ossification occurs frequently. The caseated and calcified center may be resorbed. In 39 patients over the age of 40, tubercle bacilli were not demonstrable histologically. It is therefore unlikely that a primary intestinal focus may be the source of a later endoglandular exacerbation. In a total of 2,500 autopsies the residues of a primary intestinal infection were found in 4 per cent of the cases.

MAX PINNER.

CAVERNOUS PULMONARY PHTHISIS WITHOUT INVOLVEMENT OF THE APEXES. B. ELIAS, Beitr. z. Klin. d. Tuberk. **80**:175, 1932.

The literature contains reports of twenty-one or twenty-two cases of pulmonary tuberculosis with reports of autopsy in which absence of tuberculous involvement of the apexes has been demonstrated. These cases are important in proving that pulmonary tuberculosis in the adult does not always start in the apexes. The case reported in detail in the present paper was one of acute pulmonary tuberculosis in a man, 30 years old. At autopsy, no tuberculous lesions could be discovered in the apexes, and this fact was confirmed by serial histologic sections.

MAX PINNER.

THE ACTION OF RADIUM RAYS ON TUBERCLE BACILLI IN CULTURE. A. DIMTZA and O. SCHÜRCH, Beitr. z. Klin. d. Tuberk. **80**:225, 1932.

Tubercle bacilli are little sensitive to Gamma rays, while Beta rays are highly injurious to them. These conclusions are based on experiments with tubercle bacilli on egg medium into which were stuck glass needles containing radium emanation.

MAX PINNER.

TYPHOID AND PARATYPHOID INFECTIONS IN RABBITS. ALICE WALDMANN, Zentralbl. f. Bakt. (Abt. 1) **120**:52, 1931.

Intraduodenal introduction of living cultures of Eberthella typhi Salmonella, paratyphi and S. enteritidi into rabbits caused enteritis and areas of necrosis with histologic proliferation in the small intestine and mesenteric lymph nodes. S. enteritidis was the most toxic, S. paratyphi the next and E. typhi the least. With S. enteritidis, especially, there were marked reactions in the lymphoid follicles, with prominent secondary nodules and with histiocytic proliferations and leukocytic and polyblastic infiltrations. The mesenteric lymph nodes showed hyperplasia of reticular cells and necrotic nodules in the cortical substance. The changes in the liver and spleen consisted of a slight swelling of the Kupffer and reticular cells.

Intravenous injections of similar quantities of these micro-organisms caused no detectable effects.

PAUL R. CANNON.

MIXED AND SECONDARY INFECTION. H. KAWAMURA, Zentralbl. f. Bakt. (Abt. 1) **120**:59, 1931.

When mice were infected with two different strains of spirochetes of recurrent fever, the effect was in some instances a stimulation of growth of one strain and in other instances a repression. The effect was not a direct bacterial antagonism, since both strains were frequently found simultaneously in the blood, and their simultaneous persistence in the brain was not influenced. When mice were infected with trypanosomes of Nagana and with spirochetes of recurrent fever, there was a prolongation of the trypanosome infection, similar to that obtained by splenectomy or by "blockade." The injection of serum or the transplantation of organs from mice or guinea-pigs infected with spirochetes into trypanosome-infected mice had no influence on the course of the trypanosomial infection.

The repressing effect of the spirochetal on the trypanosomal infection is due to a nonspecific stimulation of the defensive mechanisms of the host.

PAUL R. CANNON.

APPEARANCE AND SIGNIFICANCE OF BACTEREMIA IN DIPHTHERIA. T. U. SIMIČ, Zentralbl. f. Bakt. (Abt. 1) **120**:385, 1931.

In seven patients dying from diphtheria Simič recovered diphtheria bacilli from the blood of the heart or from the lungs, vena cava, spleen or pericardium. On the basis of experimental studies he concludes that the dissemination of the bacilli through the blood stream depends on their virulence and on their ability to grow and form toxin rapidly, as well as on the individual dispositions of the host. Vaccination of guinea-pigs with the anatoxin of Ramon protected against all strains of diphtheria bacilli in his possession.

PAUL R. CANNON.

BIOLOGY OF STREPTOCOCCUS VIRIDANS IN ENDOCARDITIS LENTA. B. JURUKOFF, Zentralbl. f. Bakt. (Abt. 1) **120**:392, 1931.

Seven strains of Str. viridans recovered from the blood streams of patients with endocarditis lenta were identical morphologically and culturally. Their properties remained stable and corresponded to those of Str. viridans of Schottmüller. Jurukoff concludes that this streptococcus is the cause of endocarditis lenta.

PAUL R. CANNON.

DIFFERENT VARIETIES OF TYPHUS FEVER AND THEIR BIOLOGIC DIFFERENTIATION. R. KRAUS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:353, 1932.

The adaption of the strains of virus to the different hosts results in changes of their pathogenicity and of their antigenic qualities. This explains the presence of identical biologic reactions in entirely different clinical syndromes, and, on the other hand, the absence of such reactions in clinically related symptom complexes.

I. DAVIDSOHN.

ACTION OF THE TOXIN OF BACILLUS DYSENTERIAE. G. S. BARG, Ztschr. f. Immunitätsforsch. u. exper. Therap. **74**:372, 1932.

The toxin of Shigella dysenteriae had an inhibiting effect on the growth of tissue cultures, but only in a considerable concentration (about 33 per cent). The effect was the same on the tissues of animals known to be indifferent or susceptible to the organism. Heating of the toxin did not influence the effect. The action is mild compared with the known powerful inhibiting effect of diphtheria toxin. The results are contrasted with the marked general toxicity of the dysentery toxin when injected intravenously into rabbits. It seems that the necrotic changes in the intestines of susceptible animals are not due to a direct local effect of the toxin.

I. DAVIDSOHN.

THE PRESENCE OF TUBERCLE BACILLI IN HEN'S EGGS. S. LICHTENSTEIN, Ztschr. f. Tuberk. **64**:245, 1932.

Live and virulent tubercle bacilli were found in 0.38 per cent of 525 market eggs, and in 0.46 per cent of 216 eggs from hens living in tuberculous surroundings. In eggs artificially infected with avian bacilli, tubercle bacilli could be isolated in pure culture up to the hundred and sixty-seventh day, and they were demonstrable morphologically up to the one hundred and ninety-eighth day.

MAX PINNER.

TUBERCLE BACILLURIA AS A MANIFESTATION OF SYSTEMIC INFECTION IN PULMONARY TUBERCULOSIS. H. DEIST, Ztschr. f. Tuberk. **64**:256, 1932.

Eleven rabbits were inoculated with tubercle bacilli, and their urine was injected repeatedly into guinea-pigs. Of 105 guinea-pigs, 29.5 per cent developed signs of tuberculosis. (It is not clear how many of these animals had spontaneous tuberculosis, which admittedly occurred.) By the method described, tubercle bacilli were demonstrated in the urine of each rabbit at some time. Similar experiments were performed with the urine of 15 patients with pulmonary tuberculosis who presented no indication of urogenital infection. The results demonstrated that seven of them had tubercle bacilli in the urine. It is concluded that tubercle bacilli may pass through the kidney without tuberculous foci in the renal system, and that in pulmonary tuberculosis, tubercle bacilli must at times enter the general circulation.

MAX PINNER.

ISOLATION OF TUBERCLE BACILLI FROM THE BLOOD ACCORDING TO LÖWENSTEIN. P. KALLÓS, Ztschr. f. Tuberk. **64**:371, 1932.

It was ascertained that artificially infected blood yields good growth when treated according to Löwenstein's method. The failure to confirm the findings of Löwenstein and his co-workers can therefore not be explained by technical errors. Thirty-four patients with cutaneous tuberculosis and tuberculids were examined two or three times each. All yielded completely negative results. It is pointed out that these negative results are in accord with all the work published, with the exception of that reported by Löwenstein and his co-workers. The occurrence of atypical acid-fast organisms may be an important factor in explaining the discrepancy between Löwenstein's work and that of others.

MAX PINNER.

EXTIRPATION OF SPLEEN AND BLOCKADE OF THE RETICULO-ENDOTHELIAL SYSTEM AND ITS INFLUENCE ON TUBERCULOSIS. I. M. SUBIN, Ztschr. f. Tuberk. **64**:436, 1932.

Splenectomy exerts an unfavorable influence on the course of experimental tuberculosis in rabbits. The influence of blockade is dependent on the dosage of the injected colloidal substances. Small amounts which activate the reticulo-endothelial system act favorably; large amounts act unfavorably.

MAX PINNER.

Immunology

IMMUNITY TESTS IN YELLOW FEVER. T. P. HUGHES and W. A. SAWYER, J. A. M. A. **99**:978, 1932.

The yellow fever protection test in mice, as an example of the immunity tests used in epidemiologic studies, was investigated for specificity and reliability. Serums of persons in Canada and China who could never have been exposed to yellow fever virus were tested for protective power against this virus. In only one instance was there any demonstrable nonspecific protective power, and it was insufficient in amount to give protection with regularity. The test was found to be highly specific and useful as an indicator of past infection.

AUTHORS' SUMMARY.

EFFECT OF ALLERGY IN EXPERIMENTAL TUBERCULOUS MENINGITIS. C. G. BURN and K. H. FINLEY, J. Exper. Med. **56**:203, 1932.

When living or dead tuberculosis bacilli and their products are placed in direct contact with the leptomeninges of hypersensitive (tuberculous) animals, there is a definite clinical and pathologic response. The clinical response is characterized by an onset of weakness, twitchings, convulsions and death of the animal within from six to twelve hours. Histologically, the central nervous system shows an extensive polymorphonuclear exudate distributed throughout the subarachnoid spaces of the brain and extending into the perivascular spaces. The intensity of the response is directly proportional to the quantity of visceral tuberculosis or to the dose of tuberculin employed. When small quantities of tuberculin are employed so as to permit the animal to survive longer than twenty-four hours, an exudate is found in the sulci and at the base of the brain which is characterized by small lymphocytes. The nontuberculous animals when inoculated with tuberculin or tuberculosis bacilli revealed no clinical or pathologic response. The tuberculous animals, on the other hand, when inoculated with glycerin broth, always responded by a definite but slight polymorphonuclear exudate. The possible relationship of the allergic state to postinfectious complications of the central nervous system is discussed.

AUTHORS' SUMMARY.

THE IMMUNOLOGICAL BEHAVIOUR OF THE SECOND PROTEIN (LIVETIN) OF HEN'S EGG YOLK. T. H. JUKES and H. D. KAY, J. Exper. Med. **56**:469, 1932.

A comparison of the livetin of egg yolk and serum globulin of the common fowl, by anaphylactic and precipitin tests, indicates that the two proteins are closely related, if not identical. The complement fixation test does not give the same result but indicates a difference between the two proteins. The complement fixation test also shows differences between serum euglobulin and serum pseudoglobulin when these proteins, prepared with considerable care, cannot be distinguished by either anaphylactic or precipitin tests.

AUTHORS' SUMMARY.

IMMUNIZATION AGAINST EPIDEMIC POLIOMYELITIS. M. BRODIE, J. Exper. Med. **56**:493 and 507, 1932.

The author concludes that a single intracutaneous inoculation with a subinfective dose of the virus of poliomyelitis produces considerable immunity. Combinations of virus and serum produce an appreciable immunity, provided just sufficient serum is used to protect the animal from paralysis. If there is an excess of serum, the degree of immunity is considerably reduced.

He also found that in a good proportion of urban adults the serum contained substances that neutralized the poliomyelitis virus; the incidence of potent serum was greater among contacts than among noncontacts.

THE QUANTITATIVE ACTION OF A SPECIFIC ENZYME IN TYPE III PNEUMOCOCCUS DERMAL INFECTION IN RABBITS. K. GOODNER and R. DUBOS, J. Exper. Med. **56**:521, 1932.

The enzyme which specifically decomposes the capsular polysaccharide of Pneumococcus type III must be used in certain definite amounts in order to bring about the recovery of rabbits infected intradermally with this organism. The experiments reported in this paper indicate that the minimal amounts of enzyme required bear a definite relationship to the severity of the infection, as gaged by the number of pneumococci present in the circulating blood.

AUTHORS' SUMMARY.

PRESENCE IN ANTIPNEUMOCOCCIC SERUM OF A NONANTIBACTERIAL THERAPEUTIC FACTOR. A. B. SABIN, J. Exper. Med. **56**:531, 1932.

Antipneumococcic serum contains, in addition to antibodies against the various bacterial constituents, a nonantibacterial therapeutic factor. The nonantibacterial factor has been separated from the antibacterial antibodies by absorption with homologous, heat-killed, virulent pneumococci. The nonantibacterial factor is not type-specific; it is probably specie-specific; it is not neutralized by concentrated pneumococcus broth culture filtrate. The therapeutic effect of the nonantibacterial factor was demonstrated on the experimental, dermal pneumococcus infection of rabbits; this effect is demonstrable when it is administered per se or in conjunction with a certain subeffective dose of serum. Evidence is presented for the assumption that the ultimate outcome of pneumococcus infection depends to a considerable extent on this nonantibacterial factor.

AUTHOR'S SUMMARY.

IMMUNITY TO SWINE INFLUENZA. R. E. SHOPE, J. Exper. Med. **56**:575, 1932.

Of the two etiologic components of swine influenza, only the filtrable virus possessed immunizing properties. H. influenzae-suis, while essential to the production of the disease, played only a secondary and contributory rôle and alone conferred no immunity. Serum of swine convalescent from the filtrate disease neutralized the swine influenza etiologic complex of organism and virus. Intramuscularly administered swine influenza virus was incapable of inducing illness, but did render hogs immune to swine influenza. It is suggested that a specific relationship, as regards infectivity, exists between the swine influenza virus and the tissues of the respiratory tract.

AUTHOR'S SUMMARY.

BLOOD GROUPING IN POLIOMYELITIS. C. W. JUNGEBLUT and L. W. SMITH, J. Immunol. **23**:35, 1932.

The incidence of blood groups in cases of paralytic poliomyelitis occurring in patients under 5 years of age shows no radical deviation from the normal figures. Above that age there is a distinct diminution in the B type of blood and a slight increase in O. This shift in the distribution of blood groups becomes progressively more noticeable with the advance in the age at onset, coincident with the drop in general morbidity. The largest number of deaths after 5 years of age occurred in patients other than those with the B type of blood, with a predominance of those with the A type in the period from five to ten years, and of those with the O type in the patients over 10 years The prevalence of poliomyelitis in different parts of the world appears, with certain exceptions, to diminish in direct proportion to the increase in group B blood and the decrease in groups A and O among the human races. This relative racial insusceptibility seems to occur independently of climatic factors. Not more than one third of the six human convalescent serums belonging to blood group A, and not more than about half of thirteen serums belonging to blood groups O and AB were capable of neutralizing the virus of poliomyelitis in vitro. On the other hand, six of seven B serums were found to possess this property. These results may be useful in pointing the way to a more judicial selection of suitable donors in the collection of convalescent serums for therapeutic purposes.

AUTHORS' SUMMARY.

SEROLOGICAL DIVERSITY AMONG MENINGOCOCCI. S. E. BRANHAM, J. Immunol. **23**:49, 1932.

Accurate "typing" of meningococci is often a laborious process. This is due chiefly to the following factors: lack of specificity among meningococci, lack of specificity in "typing" serum, lack of antigenic stability in meningococci and failure to do the "typing" soon after isolation. It is frequently necessary to use a combination of many methods in order to place some strains in their respective serologic groups. The relative proportion of meningococci which fall into the four groups

of Gordon and Murray has not been the same during the outbreaks from 1927 to 1930 as during those from 1915 to 1918. More than 80 per cent of strains studied have been placed in the I and III groups, the relation of which to each other is so intimate that the classification of many strains as I or III has been difficult. Intensive study of the serologic pattern of meningococci is important in respect to the choice of strains to use in making therapeutic serums, and in choosing standard "type" strains. The value of expending the amount of time and labor necessary for the satisfactory classification of complex strains in some routine work may be questioned.

AUTHOR'S SUMMARY.

HEREDITY OF THE AGGLUTINOGENS M AND N OF LANDSTEINER AND LEVINE. A. S. WIENER, S. ROTHBERG and S. A. FOX, J. Immunol. **23**:63, 1932.

The bloods of 461 mothers with 495 children were examined for the agglutinogens M and N of Landsteiner and Levine. Not once were the combinations, M + N — mother with M — N + child, or M — N + mother with M + N — child, found. The results fully confirm the theory of Landsteiner and Levine. The medicolegal application of the agglutinogens M and N is fully justified at present.

AUTHORS' SUMMARY.

PASSIVE TRANSFERENCE OF URTICARIAL HYPERSENSITIVENESS TO FUNGUS EXTRACTS. M. B. SULZBERGER, J. Immunol. **23**:73, 1932.

Reagins which sensitized the skin to extracts of pathogenic fungi of the Trichophyton or Achorion group did not sensitize to Oïdium (Monilia) extracts. This coincides with previous observations which point to the immunologic relationship of the Trichophyton, Achorion and Microsporon fungi and to their immunologic differentiation from Monilia. Three of the seven patients used as test persons did not accept passive sensitization with these reagins. Only these three had previously been infected (*experimenti causa*) with an Achorion fungus, and had received injections of trichophytin prior to their employment in my experiments.

AUTHOR'S SUMMARY.

AGGLUTININ RESPONSES TO TYPHOID-PARATYPHOID VACCINATION. S. MUDD, J. Immunol. **23**:81, 1932.

Recent claims that prophylactic vaccination against typhoid and paratyphoid fever is not effective because of the absence of somatic antibodies in the serums of vaccinated persons have not been substantiated; somatic antibodies could be detected with proper reagents after administration of a proper vaccine. A vaccine which failed to prevent small outbreaks of typhoid and paratyhyoid B infection in a state hospital was found to be deficient in production of somatic agglutinins, and its bacterial count was far below specifications. A convenient detector of H agglutination, namely, chloroform-treated bacilli, is described.

AUTHOR'S SUMMARY.

AGGLUTINATION TEST IN THE DIAGNOSIS OF INFECTIOUS ABORTION IN CATTLE. C. R. DONHAM and C. P. FITCH, J. Infect. Dis. **51**:162, 1932.

There were discrepancies in the results obtained by a single technician testing duplicate samples of bovine serums with the same rapid antigens, and in the results obtained by different technicians using the same serums and rapid antigens. Commercial rapid antigens were found to be unequal in sensitivity. Most discrepancies in the results of the rapid agglutination test and in the results of this method in comparison with those of the test tube method were in serums of from low to medium agglutinin content. The discrepancies in the results of the rapid agglutination test concerned only from 5 to 6 per cent of the bovine serums received in routine. It was not possible to adjust satisfactorily the sensitivity of the rapid-test antigen by varying the bacterial concentration. Factors other than bacterial concentration

are involved. The change in the bacterial concentration of the rapid-test antigen by the addition of various amounts of agglutinating serum to the antigen and the maintenance of uniform total volumes of the serum-antigen mixtures were not of value in the technic of the rapid agglutination test. There was no significant difference in the sensitivity of rapid-test Brucella abortus antigens containing concentrations of sodium chloride varying from 0.85 to 15 per cent. The extent of visible agglutination after from two to fourteen minutes in the rapid test was dependent, in part, on the dilution of the agglutinating serum and antigen with saline solution in the antigen, an increase in the amount of saline solution resulting in a lower agglutination titer. Also the less the bacterial concentration (see the second section of this paper), within certain limits, the higher was the agglutination titer. The rapid test antigen is composed of two materials, bacteria and saline solution, which exert an influence on the agglutination titer, one working in opposition to the other.

AUTHORS' SUMMARY.

LIPOID ANTIGENS AND ANTIBODIES IN MALIGNANCY AND PREGNANCY. HERMANN LEHMANN-FACIUS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:217, 1932.

In rabbits into which the boiled albumin fractions of serum from cancerous patients were injected, immune serum produced fixed complement with alcoholic extracts of cancerous tissue and of cancerous serums in higher dilutions than with extracts of normal tissues and of normal serums. Alcoholic extracts of cancerous serums absorbed the antibodies from a proper anticancerous serum produced in rabbits, while alcoholic extracts of normal serum had no such effect. This proves, according to Lehmann-Facius, the presence of lipoid antigens in the blood serum of patients with malignant tumors. Lipoid antibodies in the serum of cancerous patients and of pregnant women, demonstrated by complement-fixation with cholesterolized alcoholic cancer extracts, according to the method of Hirszfeld and Halber, gave only a small percentage of positive results (thirty-three of ninety-four). The nonspecificity of the reaction is shown by the finding that the best antigenic extracts are obtained from intestines and sarcoma of the mouse. In some cases, but not always, the cholesterol is the active antigenic reaction. Nonspecific reactions were obtained with serums of patients with acute infectious diseases. The albumin fraction of the serum contains the substance reacting in the complement-fixation test. Syphilitic serums also give complement-fixation tests with the cholesterized antigens used for the demonstration of tumor lipoid antibodies. It was possible to remove the syphilitic complement-fixing antibodies by heating the diluted serums for one-half hour at 65 C. Such heating of the serums also limits their reactivity with many of the cholesterolized alcoholic extracts and with cholesterol, and thus increases the specificity of the reaction. Lecithinized ether extracts of intestines and sarcoma of the mouse proved to be the most efficient antigen also with heated serum. Serums of patients with tumors and of pregnant women could not be differentiated by this test. The thermostable lipoid antibodies in the serums of patients with tumors and of pregnant women are heterophilic in nature, but not identical with the Forssman antibodies, as shown by reactions with the organs of guinea-pigs. The corresponding antigen or antigens are present in human and animal neoplasms, in the pregnant woman and in certain normal tissues (intestines of the mouse).

I. DAVIDSOHN.

GROUP SPECIFIC DIFFERENTIATION OF HUMAN ORGANS. I. L. KRITSCHEWSKI and M. M. BASKIN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:284, 1932.

Kritschewski and Baskin have tried the technic of Thomsen (*Acta path. et microbiol. Scandinav.*, supp. 5, p. 57, 1930) for the demonstration of group specific substances by observing the inhibition of agglutination of red blood cells by the corresponding iso-agglutinating serum, to which aqueous organ extracts were previously added. They found it not reliable and inferior to the previously published technic of Kritschewski and his associates.

I. DAVIDSOHN.

AGGLUTINATION AND HEMOLYSIS IN THE FISH. LADISLAUS VON TOTH, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:277, 1932.

Blood groups could not be found in Cyprinus carpio; however, only 280 combinations were studied in 25 of a possible total of 600 animals. It has not been established whether the absence of group reactions was due to the lack of iso-agglutinins or of iso-agglutinogens. Hetero-agglutination was studied in 17 species of fish and demonstrated among some of them. The difference in the intensity of the agglutination and in the speed of the reaction is due to properties of the serum, while the red blood cells show only insignificant variations in that respect. The study of heterohemolysis in the same animals confirmed the observation made by Aschenheim in 1909 that the natural hemolytic qualities of a serum are inversely proportionate to the ability of the red cells of the same serum to become hemolysed by the other serums.

I. DAVIDSOHN.

PRIMARY TOXICITY OF THE LENS AND THE ANAPHYLACTIC EXPERIMENT. REINHARD BRAUN, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:326, 1932.

Aqueous extracts of fresh and of dried lenses of the hog and of human lenses with a cataract produced contractions in the Dale preparations of uterine horns of nonsensitized guinea-pigs. No difference was noted in the effect if uteri of previously sensitized animals were used. Dried lenses of guinea-pigs had a similar effect on the guinea-pig uterus, but only very faint and indistinct reactions were observed when the lens of the animal was permitted to act on the preparation of its own uterus. Desensitization by preliminary addition of the lens substance to the solution was not observed. Injection of the aqueous extracts into living guinea-pigs produced mild toxic symptoms, resembling manifestations of slight anaphylaxis. Braun believes that the results throw considerable doubt on the reports in which the anaphylactic experiment was used for demonstration of organ specificity of the lens, and of the possibility of isosensitization and autosensitization with the lens.

I. DAVIDSOHN.

EFFECT OF BACTERIOPHAGE ON THE FORSSMAN AND HUMAN BLOOD ANTIGENS IN B. PARATYPHOID B AND IN B. DYSENTERIAE SHIGA. M. EISLER and A. F. HOWARD, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:366, 1932.

A polyvalent bacteriophage caused a slight diminution of the A-receptor-like fraction present in some strains of B. paratyphoid B. The diminution was much weaker than the one previously reported for B. dysenteriae Shiga. The difference in the effect is explained by the qualities of the phage employed in the experiments.

I. DAVIDSOHN.

ACTION OF EXTRACTS OF FECES ON BACTERIAL BLOOD ANTIGENS AND ON HUMAN ERYTHROCYTES. M. EISLER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:418, 1932.

Schiff and others have shown that suspensions of human feces are able to destroy blood-group specific antigenic properties of some human secretions and of blood. The B. paratyphoid B and B. dysenteriae Shiga contain, in addition to the Forssman antigen, an antigenic substance which is related to human red blood cells. Eisler applied the results of Schiff in the study of the effect of fecal extracts to the aforementioned bacteria and to human red blood cells. While the antigenic properties of the bacteria were left intact, human red blood cells became modified in a manner similar to that which occurs in the so-called Thomsen phenomenon; i. e., they became agglutinable by serums of their own group and by other serums which heretofore were not able to agglutinate them. The agglutination was not so marked as it is when brought about under the influence of the bacteria M. and J. Filtrates of the more important fecal bacteria did not modify the red cells, as did extracts of whole feces.

I. DAVIDSOHN.

EFFECT OF INTRACUTANEOUS INJECTIONS OF TYPHOID VACCINE IN TYPHOID FEVER. RUDOLF REITLER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:471, 1932.

Five or six daily intracutaneous injections of 20,000 killed bacilli were given, and in some cases were followed by another course after a five day interval. Controls of healthy persons and of patients suffering from various other diseases were employed. The local skin reactions were different in the typhoid fever patients and in the controls. Focal reactions and general reactions consisting of an increase in the agglutinin titer and in the pronounced changes in the white blood cell picture were noted. The latter differed greatly, both quantitatively and qualitatively, from the changes seen in the nontyphoid controls. Reitler suggests a formula which expresses the relations of the various components of the white blood cell picture. One hundred cases of typhoid fever were treated with the vaccine, together with one hundred control cases of the disease. A shortening of the duration of the disease and a decrease in the number of recurrences and of deaths were observed in the treated patients.

I. DAVIDSOHN.

IMMUNOLOGIC PROPERTIES OF NORMAL SERUM. SALVATORE MAUGERI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:473, 1932.

Friedberger and his associates have shown that the development of diphtheria antitoxin in man and of antisheep hemolysin in man and in the rabbit are a result of constitutional maturation and not a response to a contact with the antigens. On the other hand, the titer of the complement in the new-born guinea-pig is as high as in the adult animal. In the present investigation rats and guinea-pigs had no complement, nor did antisheep hemolysin at birth and for a considerable period afterward. Both were present in adult animals. In man the complement was found in a similar titer as in the adult, while the antisheep hemolysin appeared only after a period of growth.

I. DAVIDSOHN.

SPECIES AGGLUTINATION: I. V. N. KRAINSKAJA-IGNATOWA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:489, 1932.

Krainskaja-Ignatowa was able to demonstrate agglutination of red blood cells by the serums of a large number of animal species and of man; among the latter, by some concerning which there had been previous reports to the contrary. The agglutination is species-specific; i. e., when the serum is treated with the red blood cells or tissues of a species, agglutinins for only that species are removed, and those for other species are left intact. Krainskaja-Ignatowa favors the use of the term "species agglutination" instead of the older word "hetero-agglutination," because the latter implies lack of specificity of the phenomenon, as had been previously assumed.

I. DAVIDSOHN.

HEMAGGLUTINATION AND HEMOLYSIS IN MACACUS CYNOMOLGUS AND SIMIA RHESUS. WERNER FISCHER and GERTRAUDIS KLINKHART, Ztschr. f. Immunitätsforsch. u. exper. Therap. **75**:513, 1932.

The red blood cells of the Macacus and Rhesus monkeys are agglutinated by human serums of all groups, and their serums in turn agglutinate erythrocytes of all human groups. The agglutination by human serums is brought about by hetero-agglutinins, different from the iso-agglutinins anti-A and anti-B. The red blood cells and serums of the lower order of monkeys under consideration do not possess the qualities of the human group, nor do their serums have normal iso-antibodies, but by means of cross-immunization one receptor could be demonstrated in the red blood cells of twenty-six of thirty monkeys, permitting their separation into two groups. Iso-agglutinins and isohemolysines have been produced by cross-immunization, but only in the Rhesus monkeys. Immunization with human red cells of type O stimulated the development of only species-specific, not of group-specific, antihuman hetero-agglutinins.

I. DAVIDSOHN.

Tumors

EFFECT OF CARBON MONOXIDE AND OTHER AGENTS UPON THE RATE OF TUMOUR GROWTH. J. A. CAMPBELL, J. Path. & Bact. **35**:379, 1932.

It is shown that breathing carbon monoxide (up to 0.28 per cent) for prolonged periods has a marked retarding effect on the rate of growth of mouse carcinoma 63 and increases duration of active life after inoculation. No such effect was observed with the Rous fowl sarcoma. Some experiments with Fischer-Wasels' gas therapy and others with the "growth-retarding hormone" gave no evidence of inhibitory effects on Rous fowl sarcoma and mouse carcinoma 63.

AUTHOR'S SUMMARY.

TRANSMISSION OF FOWL-TUMOURS TO PHEASANTS. C. H. ANDREWES, J. Path. & Bact. **35**:407, 1932.

Both cells and filtrates of Rous sarcoma no. 1 have produced fatal progressive tumors in the large majority of inoculated pheasants. Metastases have occurred in nearly all tumor-bearing pheasants, particularly in the lungs. Serial transfer of Rous no. 1 sarcoma from pheasant to pheasant has, however, proved unexpectedly difficult. Cells of Fujinami's fowl myxosarcoma and of the RF 4 spindle cell sarcoma also produce progressive tumors in pheasants. Three other filtrable fowl tumors, the spindle cell sarcoma BS 1, the fibrosarcoma RF 11 and the endothelioma MH 2, have so far failed to grow progressively in pheasants.

AUTHOR'S SUMMARY.

TUMOUR IMMUNITY. T. LUMSDEN, J. Path. & Bact. **35**:441, 1932.

Forty-seven mice suffering from spontaneous carcinoma were treated by inoculating the euglobulin fraction of an anticancer (sheep) serum into the area of the tumor. Some days later the tumor was removed, in order to prevent death from absorption of too much necrotic material. In forty of the mice an autoplast was implanted. Forty control mice were similarly treated, except that in this case, the euglobulin fraction was made from the serum of a normal sheep or horse, and not from an animal that had been immunized against cancer. In all the controls, autoplasty was performed. The autoplast failed to grow in thirty-one (77.5 per cent) of the forty antiserum-treated mice, while in the forty controls only one autoplast (2.5 per cent) failed. In seven of the antiserum-treated mice, no autoplast was implanted, but recurrence did not take place after incomplete excision. Of the forty-seven antiserum-treated cancerous mice, thirty-four (74.7 per cent) were cured, while in thirteen (25.3 per cent) either the cancer recurred (four mice) or the autoplast grew progressively (nine mice). The euglobulin fractions of anticancer serums are very labile and must be freshly prepared for each treatment. These experiments indicate that it is possible to evoke or increase resistance in mice against their own spontaneously arising cancerous tumors. Antiserum treatment is not at present applicable to human cancer.

AUTHOR'S SUMMARY.

PRIMARY ROUND-CELLED SARCOMA OF THE PERITONEUM. T. F. HEWER, J. Path. & Bact. **35**:727, 1932.

A man, aged 40, gave a history of abdominal pain and constipation preceded for a few hours by faintness and malaise; on the sixth day he showed signs of paraplegia. A diagnosis of paraplegia due to compression by a tumor in the lower dorsal region was made. During the ten days before death the patient had occasional convulsions and passed into a uremic state. Postmortem examination revealed extensive infiltration of the peritoneum, ureters and renal pelves with a pale gray tumor. An intraspinal (extradural) metastasis was responsible for the paraplegia.

The uremic termination was evidently due to obstruction of the kidney tubules by the tumor lining the pelves. The tumor in this case differs from the three previously described in that it is a pure round cell sarcoma, with a rapid course and a metastasis in the spinal canal, but even so the gross appearance is extraordinarily similar to that of the others. There would seem to be little doubt that this condition represents a true tumor of the connective tissue cells of the peritoneum and is not related in any way to the various types of retroperitoneal tumor.

AUTHOR'S SUMMARY.

OCCUPATIONAL INCIDENCE OF PRIMARY LUNG CANCER. WILLIAM BROCKBANK, Quart. J. Med. **1**:31, 1932.

An investigation of the occupations of 898 persons with primary cancer of the lung, reports of whose cases were collected from the literature, suggests that the laboring classes predominate, although no one occupation stands out. The poison gas of war, smoking of tobacco, road dust and fumes of motor cars are all possible etiologic factors. The occupations of sixty-two were investigated in some detail. Nine (14.5 per cent) seemed to have definitely dusty jobs, and eighteen (29 per cent) worked among gases and fumes. Four were badly gassed in the war, and nine smoked tobacco excessively. A plea is made for more detailed investigation of the occupations of patients suffering from this disease.

AUTHOR'S SUMMARY.

OSSEOUS TUMORS WITH THYROID STRUCTURE. P. MOIROUD and J. COTTALORDA, Ann. d'anat. path. **9**:123, 1932.

The authors raise *de novo* the question of whether or not the apparently normal thyroid gland metastasizes to distant organs. They report a case of a woman, aged 57, who seven years previously had a fracture of the radius following a slight trauma. Roentgen examination showed that the bone was broken as a result of an invasion by a neoplasm which on histologic examination revealed the structure of a normal thyroid. Following the removal of the arm, the patient was observed for seven years, without signs of malignancy of the thyroid gland or of any other part of the body appearing.

The authors quote a number of authorities, whose views they share, who are of the opinion that there is no such thing as a metastatic goiter, but that there are latent cancers of the thyroid gland of slow evolution. The first manifestation of these tumors is that of a metastasis to the bone or viscera.

B. M. FRIED.

CANCER IN COCHIN CHINA. J. BABLET, Ann. de Inst. Pasteur **48**:594, 1932.

A statistical study of cancer among the Annamites of the province of Tonkin in French Indo-China led the author to the following conclusions: The results of his inquiries in Tonkin confirmed and completed the information recorded in Cochin China as regards the susceptibility of the Annamite race to cancer, the relative proportion of tumors observed, and the age and sex of the victims. 1. In a general way, one may say that in the vast alluvial plain which constitutes the delta of the Rouge in Tonkin, the incidence of cancer differs little from that observed in temperate countries. 2. Histologic examination allows the classification of tumors seen within the usual limits of the classic groups which rarely merit special description. 3. The proportion of sarcomas relative to epithelial tumors is distinctly increased. 4. Certain localizations (such as the neck and buccal cavity) are abnormally numerous in the Tonkinese; cancers of the digestive tract, however, are rare, and cancer of the skin is also less frequent than in Europe. 5. Both sexes of Annamites appear to be equally attacked. 6. The age of victims is notably lower, in part owing to the frequency of sarcoma and in part to the precocity of epithelioma.

M. L. MARSHALL.

MUCUS-SECRETING CELLS IN BENIGN EPITHELIOMAS OF THE SKIN. H. ŠIKL, Frankfurt. Ztschr. f. Path. **43**:1, 1932.

The author describes two cases. Because of the presence of cylindric cells with small cysts in the midst of an epithelioma in the first case, the erroneous diagnosis of metastatic carcinoma in a benign epithelioma (collision tumor) was made. That the patient was in good health over a period of four years indicated that originally there was no malignancy. The second tumor was a benign cyst of the corium with squamous cells and keratinization and also mucus-secreting goblet cells. The various possible explanations for the occurrence of these tumors are discussed.

O. SAPHIR.

SEROLOGY AND CANCER RESEARCH. H. LEHMANN-FACIUS, Frankfurt. Ztschr. f. Path. **43**:19, 1932.

The antigen of malignant tumors consists of at least three components. One is the "individual specific structure," also reacting specifically in regard to organs from which the tumor arises. The second constitutes the "species-specific, carcinoma lipoids," while the third component is the so-called "system-specific, general tumor lipoid." Because of the fact that serum taken from carcinomatous patients and serum from pregnant women show similar properties, the author believes that both types of serum contain antibodies directed against abnormal growths in general rather than that specific antibodies which are directed against the antigen of carcinomatous origin circulate in carcinomatous patients.

O. SAPHIR.

CHORIONEPITHELIOMA OF THE TESTIS WITH ABNORMAL DEVELOPMENT OF THE MALE BREAST. K. R. STORJOHANN, Frankfurt. Ztschr. f. Path. **43**:80, 1932.

A man, aged 20, for two years had a tumor of the testis and enlargement of the breasts. The tumor was removed and revealed a typical chorionepithelioma with teratoid elements. After operation, the breasts became smaller. Two months later the patient died suddenly as a result of tumor embolism and hemorrhage of the brain. There also were metastases to the lungs. The metastatic nodules revealed typical chorionepitheliomatous structures. There was second degree hypertrophy of the mammary glands. The hypophysis showed fairly well recognizable pregnancy changes. There also was a moderate adenomatous hyperplasia of the prostate and seminal vesicles.

O. SAPHIR.

MYELOMA. F. GRÖGLER, Frankfurt. Ztschr. f. Path. **43**:96, 1932.

Two cases are described: In one the condition was erythroblastoma and in the other, myeloblastoma. In the first instance, a nodule had compressed the spinal cord, resulting in paraplegia. A differentiation between myeloma and aleukemic myelosis can be made only by differentiating the type of cells of which these two growths consist.

AUTHOR'S SUMMARY.

MAMMARY DUCTS IN CARCINOMA OF THE MAMMARY GLANDS. W. SCHOLZ, Frankfurt. Ztschr. f. Path. **43**:102, 1932.

In twenty-two instances of carcinoma of the mammary glands, the ducts were dilated. In six of these tumors the ducts revealed an epithelial cell proliferation. The ducts were also dilated in nine instances in portions of the breast which were not involved by the carcinoma. Since the carcinoma was also found in places which did not reveal such a dilatation, the author does not believe that the accumulations of the secreted material within the dilated ducts alone could be regarded as the causative agent of the carcinoma. There might also be a relation between the obliteration of the ducts and the formation of the carcinoma. Because of the

fact that obliteration of ducts occurs only after loss of the epithelium, and because such a loss is the result of an inflammation, it is also possible that inflammation plays a contributory rôle in the development of carcinoma.

O. SAPHIR.

EPIDERMOIDAL CHOLESTEATOMA IN THE CRANIAL CAVITY OF A HORSE. G. PALLASKE, Frankfurt. Ztschr. f. Path. **43**:126, 1932.

In an article summarizing the subject, the author states that such tumors in the horse are never found in the region of the base of the skull but are always dorsally located. They are found between the cranium and the dura in connection with the dura, possibly extending also intradurally. This location is in contradistinction to the findings in man in whom these tumors are found always at the base of the skull.

O. SAPHIR.

DUODENAL CARCINOMA. E. RUTISHAUSER, Frankfurt. Ztschr. f. Path. **43**:133, 1932.

Six cases are described. The first revealed so-called multiple malignant adenomas of Brunner's glands, with metastases to lymph nodes, to the liver and to the peritoneum. The second, third and fourth cases showed an adenocarcinoma of the duodenum, arising in the region of the papilla of Vater which was completely destroyed. The fifth case revealed an adenocarcinoma microcysticum of the duodenum just above the papilla of Vater, while in the sixth instance an ulcerated adenocarcinoma was found surrounding the ampulla of Vater. The statistics of duodenal carcinoma are reviewed, and the various etiologic possibilities for such an occurrence are discussed.

O. SAPHIR.

SUCCESSFUL ISOLATION OF THE ROUS TUMOR AGENT BY MEANS OF CAPILLARITY. H. LOEWENTHAL, Ztschr. f. Krebsforsch. **34**:551, 1931.

In an experiment of beautiful simplicity, Loewenthal has obtained the Rous tumor agent in circumstances that would appear to answer negatively once and for all the question of its action through content of minute cellular elements. In filtrate protected from oxidation by the addition of cysteine and by the use of serum bouillon for the solvent, and to which he had added Bacillus coli, strips of sterile filter paper were dipped, and after completion of the capillary rise this paper was cut in several horizontal segments. The upper one contained no colon bacilli, but after maceration in more bouillon, on inoculation into fowls, it was found to contain the filtrable tumor-producing agent. As Loewenthal himself states, the experiment casts no light on the hypothesis of a ferment-like nature of the agent.

H. E. EGGERS.

TUMOR DIAGNOSIS BY PUNCTURE. E. P. MANNHEIM, Ztschr. f. Krebsforsch. **34**:574, 1931.

Mannheim describes a method for the diagnosis of tumors by histologic examination of material removed by puncture and aspiration with a syringe. Two principal criteria are relied on: (1) the character of individual cells, and more particularly of their nuclei, which in malignancy are usually large, diffuse, with several nucleoli frequently of unusual size, and, of course, with occasional asymmetric mitoses; (2) the character and nature of cell groupings. Comparison with other tissues from the same neighborhood is sometimes necessary. Mannheim states that he has been unable to make certain diagnoses of sarcoma by this method. He considers that the method is preferable to that of biopsy in that it is less dangerous, but recommends the latter procedure when the results of his method are uncertain.

H. E. EGGERS.

OCCUPATIONAL CARCINOMA IN ANILINE WORKERS. W. BÜTTNER, Ztschr. f. Krebsforsch. **34**:605, 1931.

Of 115 cases of cancer of the bladder in men observed at the Heidelberg University clinic, 9 developed in aniline workers. The youngest of the patients was 38 years old; the oldest, 54. The average age was 48, so that apparently these cancers develop at a somewhat earlier age than is usual for cancer. Büttner is inclined to the belief that the incidence was less than it would have been except for the war, with its loss of life among men in whom these cancers might otherwise have developed. Four of the men had worked continuously in aniline industries for from twenty to twenty-five years. Among the remainder, 1 had not been so employed for seven, 1 for twenty, 1 for nineteen years, and in 1 case the cancer appeared twenty-four years after an exposure that had lasted for fourteen months only. The average latent period was twenty-three and nine-tenths years. Of 8 a detailed history of whose employment was available, 2 had worked on fuchsin, 6 on aniline, compounds. In 1 case, the exposure was merely that incurred in loading aniline products on shipboard. Büttner believes, like almost all who have studied the subject, that the irritant material is absorbed after inhalation, and produces its cancerigenic effect after elimination, with or without modification, by the kidneys. There is evidence that, with appreciation of the danger, improved technical devices have resulted in a diminution of the incidence.

H. E. EGGERS.

THE MÜLLER TEST FOR SYPHILIS. T. M. VOGELSANG, Acta path. et microbiol. Scandinav. **9**:139, 1932.

The Müller test is made by adding serum or spinal fluid to an alcoholic extract of ox heart to which has been added a definite amount of cholesterol. It is a simple test, the result of which can be read in the course of four hours. The reading of the result is easy, except in a few cases in which there develops only a fine flocculent precipitate. Vogelsang finds that the Müller test gives a positive reaction earlier as well as longer than the Wassermann test and no more nonspecific reactions than the latter. It can be used with advantage in conjunction with the Wassermann test.

Medicolegal Pathology

TOXIC CIRRHOSIS OF THE LIVER DUE TO CINCHOPHEN. C. R. WEISS, J. A. M. A. **99**:21, 1932.

The author reports three deaths due to cinchophen compounds and reviews eighty-nine cases in the literature. His first case was that of a man, aged 26, who had taken 60 grains (4 Gm.) of "Atophan" daily for a period of three months to relieve pains in the joints. Jaundice started nine weeks after the onset of therapy, and the patient died in four days of acute yellow atrophy of the liver and red hepatization of both lungs.

The second case was that of a man, aged 65, who had also taken 60 grains of "Atophan" daily for the relief of "rheumatism" for a period of four months. Weakness and jaundice started thirteen and one-half weeks after the onset of therapy, and the patient died in five days. Autopsy revealed an acute yellow atrophy of the liver and a slight diffuse nephritis.

The third case was that of a woman, aged 64, who took 14 grains (0.9 Gm.) of "Farastan" (mono-iodo-cinchophen) daily for a period of four months, when there was an onset of weakness and jaundice. She died six days after the onset of the terminal symptoms of acute yellow atrophy of the liver.

WILLIAM FREEMAN.

KEROSENE POISONING IN CHILDREN. J. P. PRICE, J. A. M. A. **99**:214, 1932.

Four cases are presented of kerosene poisoning in children who accidentally drank the fluid, one of whom died (no autopsy). The patients were 2 years of age or younger. Methemoglobin was found in the specimen of blood in every case. The onset of symptoms was with a severe gastro-intestinal toxication.

WILLIAM FREEMAN.

TRAUMATIC RUPTURE OF THE LEFT RENAL ARTERY WITHOUT INJURY TO THE KIDNEY. P. K. EDMUNDS, J. A. M. A. **99**:467, 1932.

A white man, aged 45, fell 25 feet to the ground from a scaffolding. At operation a ruptured spleen was removed. The postoperative course was stormy, with marked hypotension. No blood was found in the patient's urine. He died on the fourth postoperative morning. Necropsy revealed a tear across the left renal artery near the junction with the aorta, with a large retroperitoneal hemorrhage. The artery was completely thrombosed. No evidence of injury to the kidney substance was found.

WILLIAM FREEMAN.

POISONING BY "EXLAX" TABLETS. M. CLEEVES, J. A. M. A. **99**:654, 1932.

A healthy American boy, 10 years of age, ate the contents of a box of "Exlax" tablets which he mistook for candy. In twenty-four hours he suffered from severe purgation; his temperature was 106 F., the pulse rate, 130. Four days later a sudden eruption of huge wheals with sharply marked, serpiginous borders appeared on the skin of the abdomen, legs and arms and disappeared the following day. Hemiplegia was present on the sixth day, with delirium and multiple petechiae on the skin of the abdomen and the feet. A failure of respiration, with death, occurred on the ninth day. Necropsy elicited many hemorrhagic areas in the intestine, kidneys, liver and brain substance. The walls of the heart showed multiple minute hemorrhagic areas and infarcts in the aortic valves. The active principle of "Exlax" is said to be phenolphthalein.

WILLIAM FREEMAN.

CINCHOPHEN POISONING IN A DIABETIC WITH INDUCTION OF HYPOGLYCEMIA. G. S. BERGER and H. H. SCHWEID, M. J. & Rec. **136**:50, 1932.

A 63 year old white woman had taken massive doses of cinchophen daily for the relief of symptoms of chronic hypertrophic arthritis; she died in a state of hypoglycemic shock. At necropsy, the liver was found to weigh 940 Gm., and subacute atrophy, cirrhosis and toxic hepatitis were considered the causes of death.

WILLIAM FREEMAN.

INJURIES OF THE STOMACH AND DUODENUM BY BLUNT VIOLENCE. G. STRASSMANN, Deutsche Ztschr. f. d. ges. gerichtl. Med. **18**:297, 1931.

This is a report of two accidental deaths caused by agricultural machines. In both cases, involving women, the clothing, caught in the machine, was forcibly and tightly wound around the body, producing severe internal injuries, but no externally visible signs. In the first instance there was a complete tearing off of the duodenum with partial laceration of the stomach and omentum. Death followed twelve hours later from hemorrhage, shock and diffuse peritonitis. Rupture of the stomach may occur either from within, on account of excessive distention, or from without, owing to external violence. If the stomach is filled with food, forceful pressure from the outside will not only directly injure this organ, but simultaneously cause a bursting of the gastric wall, owing to the greatly compressed contents of the stomach. The rupture of the stomach is then the result of two components of the same violence. The linear tearing of the mucous surface due to distention occurs chiefly along the lesser curvature and close to the cardiac opening and may involve all the layers of the gastric wall. External blunt violence

acting on the stomach and pressing it toward the spinal column may tear the gastric wall completely. Laceration of the duodenum occurs on account of a similar mechanism. In the second case, several ribs and both knee caps were fractured, and a diffusely developed pulmonary and cerebral fat embolism was found. Death followed on the fourth day from bilateral traumatic pneumonia. Since the injured woman had been transported over rough roads, the continuous, prolonged jarring of the body during transportation explains the extensive development of the fat embolism of the lungs and brain.

E. L. MILOSLAVICH.

DESQUAMATION OF THE BRONCHIAL EPITHELIUM IN NEW-BORN AND YOUNG INFANTS. K. WALCHER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **18**:305, 1931.

It is well known that cell elements that invest various inner surfaces of the body, such as the genito-urinary tract, pericardial and abdominal cavities, etc., fall off soon after death, often in large numbers. This phenomenon is observed in the bronchi and bronchioli of new-born infants and of children in the first few years, while leukocytes are totally absent. This cadaveric desquamation must be distinguished from intravital desquamation. The latter is termed desquamative bronchitis and consists of accumulations of detached ciliated epithelial cells, while mucus and leukocytic elements are sparsely present. Since desquamative bronchitis and bronchiolitis may lead to the sudden death of an infant, it possesses medico-legal significance. The etiologic factors are not yet sufficiently determined; there are, however, instances in which, besides marked hyperemia of the bronchial mucosa, innumerable bacteria (particularly pneumococci), as in a pure culture, are found. Lobular atelectasis, acute alveolar or interstitial emphysema and aspiration of the ciliated epithelium into the deeper portions of the lung down into the alveoli may occur as complications of desquamative bronchitis.

E. L. MILOSLAVICH.

SUSPECTED POISONING; DEATH FROM RHEUMATIC AORTITIS. L. BREITENECKER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **18**:312, 1931.

A 22 year old girl died suddenly after a physician had administered a patented drug for her menstrual period. The autopsy disclosed complete occlusion of the right coronary artery by a recent thrombotic process and stenosis of the main coronary ostium. The aorta showed, close above the valves, marked changes arranged in girdle-like fashion, consisting of projecting yellowish patches and bluish-appearing depressions with wrinkling of the intimal surface, thus closely resembling a supravalvular type of syphilitic mesaortitis. Similar areas were found in the aortic arch. The valvular apparatus of the heart was, however, intact. Microscopic examination detected characteristic rheumatic lesions (rheumatic aortitis), and the heart muscle contained Aschoff's bodies.

E. L. MILOSLAVICH.

FATAL POISONING WITH ANILINE. RASCHEWSKAJA, Deutsche Ztschr. f. d. ges. gerichtl. Med. **19**:23, 1932.

A laborer, aged 34, swallowed a large amount of aniline oil with suicidal intent. Death followed in six hours. The clinical signs were deep coma, with a complete absence of reflexes, no vomiting, retarded respiration, but a pulse of good quality, a gray, cyanotic appearance of the skin, methemoglobinemia, p-amidophenol in the urine and a quite pronounced xanthoprotein reaction of the blood. The autopsy observations were indistinct, except for punctate hemorrhages in the gastro-intestinal tract and a suggestive aromatic odor of the internal organs. Microscopic examination of the brain revealed vacuolization and swelling of the ganglion cells in the cortex and in the wall of the third ventricle, neuronophagia and small perivascular hemorrhages. Only one additional case has been recorded in the literature, by F. Mueller in 1887.

E. L. MILOSLAVICH.

STATISTICAL AND THEORETICAL DATA ON UNEXPECTED DEATH FROM NATURAL CAUSES. E. BRACK, Deutsche Ztschr. f. d. ges. gerichtl. Med. **19**:173, 1932.

The author presents a statistical study of six hundred cases of unexpected death, with a consideration of the anatomic changes. The general circumstances relating to the place and time of death, the position in which the dead body was found and the histories obtained from relatives, friends or other persons are presented and evaluated. Death due to cardiac lesions or circulatory disturbances highly prevailed, occurring in 60 per cent of the cases, in comparison to diseases of the brain, which were the cause of death in only 6 per cent of the total observations. Rapid changes and loss of cerebrospinal fluid may lead quite rapidly to a lethal end. In two cases, a posttraumatic injury of the head caused death by an acutely developing hydrocephalus. Instances of sudden death following heavy meals, bodily exertion and psychic trauma or excitement are mentioned.

E. L. MILOSLAVICH.

CEREBRAL APOPLEXY FOLLOWING ALCOHOLIC EXCESS. BEYREIS, Deutsche Ztschr. f. d. ges. gerichtl. Med. **19**:193, 1932.

After a drinking debauch lasting an entire day up to midnight, a man, aged 30, was found unconscious in his bed the next morning; he died early in the afternoon. Autopsy revealed a hemorrhagic destruction of the left lenticular nucleus. The cerebral vessels did not show atherosclerotic changes, and the internal organs appeared otherwise unaffected. It is assumed that the acute alcoholic intoxication caused a hemorrhage from the choroid plexus, since this tissue in lower animals was found, by experimentation, to be easily injured by alcohol.

E. L. MILOSLAVICH.

POSTTRAUMATIC ANEURYSM OF A BASAL CEREBRAL ARTERY. G. JUNGMICHEL, Deutsche Ztschr. f. d. ges. gerichtl. Med. **19**:197, 1932.

A 17 year old boy was struck on the head by a stone weighing about 2 pounds, which rendered him unconscious for a while. Death occurred about eleven weeks later, and in the interval, symptoms referable to injury of the brain were evident. Autopsy disclosed a ruptured aneurysm, the size of a small pea, of the anterior communicating artery, involving mainly its left half, without microscopic evidence of inflammatory processes. Bacteriologic examination of the blood revealed anhemolytic streptococci, to which finding, however, no causal importance is given. The etiologic and pathogenic factors of cerebral aneurysms are discussed, in general, and nineteen similar cases of traumatic origin, reported in the literature, are tabulated. In regard to the relation between trauma and basilar aneurysm, the following points have to be considered: The injury to the head must be of sufficient intensity, but not necessarily severe enough, to cause a fracture of the skull, accompanied and followed by clinical evidence of concussion; in the interval between the trauma and death, bridge symptoms indicating irritation or injury of the brain would be apparent; the examination of the body should not disclose any signs of cardiovascular diseases or the presence of miliary or racemous aneurysms.

E. L. MILOSLAVICH.

THREE CASES OF ARECALINE POISONING. L. WACHHOLZ, Deutsche Ztschr. f. d. ges. gerichtl. Med. **19**:224, 1932.

Three adult tuberculous patients received intravenously, for the purpose of treatment, an injection of a 10 per cent solution of calcium chloride. Severe symptoms of poisoning followed immediately, but the patients recovered gradually. Chemical analysis of the rest of the solution showed the presence of arecaline bromate. Arecaline, an organic base of areca catechu, is used in Germany only in veterinary practice, in the form of a bromide salt (arecalinum bromatum). The

lethal dose for a horse is 0.5 Gm. These three cases are the first ones to be reported in the literature. The arecaline salts are highly poisonous and act similarly to, but far more intensely than, muscarine or pilocarpine. Arecaline produces muscular cramps, particularly of the muscles of the extremities and of those of the neck. Other symptoms of poisoning are loss of consciousness, dyspnea and cyanosis, nausea, diarrhea, salivation, involuntary micturition, myosis and bradycardia. Death occurs from cerebral paralysis.

E. L. MILOSLAVICH.

BLOOD GROUPING WITH POLLUTED AND PHYSICALLY INFLUENCED BLOOD SAMPLES. R. WERCKMEISTER-FREUND, Deutsche Ztschr. f. d. ges. gerichtl. Med. **19**:238, 1932.

Keeping blood in a moist chamber or in an incubator at 37 C. for forty-eight hours, or in a refrigerator at 5 C. for several weeks, did not influence the group determination. However, samples of blood freely exposed to open air for several days could not be used successfully for testing. The same was true if the blood was frozen, heated or subjected to the action of ultraviolet rays or to the direct influence of sun for only three hours.

E. L. MILOSLAVICH.

DETECTION OF BARIUM IN HUMAN BONES. S. KUNOWSKI, Deutsche Ztschr. f. d. ges. gerichtl. Med. **19**:265, 1932.

Barium poisoning is increasing, and up to 1924, eleven instances in connection with roentgenographic and fluoroscopic examinations alone were recorded. Barium poisoning of animals, horses, cattle and sheep, by various plants, was first observed in the western states of this country by Crawford in 1908 ("loco-weed disease"); the soil and its products were rich in barium. Under normal conditions, human bones do not contain barium, but it may be deposited therein if the ingested food, particularly vegetables, contains barium. With the ultraviolet spectrophotographic procedure, one can easily and successfully detect barium in the ashes of bones, if it is present. If a barium-poisoned corpse is cremated, the ashes will show its presence, by the spectrographic method, but one must first eliminate the possibility that the various objects in contact with the dead body may have been contaminated with barium.

E. L. MILOSLAVICH.

BARIUM IN ANIMAL BONES. F. TIMM, Deutsche Ztschr. f. d. ges. gerichtl. Med. **19**:279, 1932.

Spectrophotographic examinations of bones of wild game (deer, hare, etc.) and of domestic animals (cattle, horse, sheep, pigs) showed the presence of infinitesimal amounts of barium, estimated to be less than 0.001 per cent. This finding, to date unrecorded in physiologic chemistry, may allow a differentiation between animal and human bones, since the latter do not normally contain barium.

E. L. MILOSLAVICH.

Technical

A METHOD FOR PROGRESSIVE SELECTIVE STAINING OF NISSL AND NUCLEAR SUBSTANCE IN NERVE CELLS. L. EINARSON, Am. J. Path. **8**:295, 1932.

Well aware of the danger and difficulties of deducing from mere similarity of dye affinities the identity of chemicobiologic relations of two tissue elements, I nevertheless believe that the positive gallocyanin reaction furnishes new evidence that the Nissl substance really contains nuclear chromatin substances. The results of the chemical, the embryologic and the experimental investigations summarized in this article, and certain characteristics of the gallocyanin reaction, definitely lend support to this view. The specific Nissl staining of the gallocyanin-chromalum is practically unaffected within a wide range of p_H of the dye solution, and also in

combination with borax in an alkaline solution it still stains the chromatin and Nissl substances feebly but definitely. This indicates that possibly some chemical affinities are involved in this special staining process. Further investigation of this point is in progress.

AUTHOR'S SUMMARY.

THE SPECIFIC INTRACUTANEOUS TEST FOR INGUINAL LYMPHOGRANULOMATOSIS. W. FREI, Klin. Wchnschr. **11**:512, 1932.

The material for the test is obtained from nontuberculous, nonsyphilitic patients with typical inguinal lymphogranulomatosis. They should not have had soft chancre. They should give a positive test for inguinal lymphogranulomatosis but not for soft chancre. The enlarged inguinal glands must have foci of softening with intact cutaneous covering. The skin is disinfected and the softened material aspirated with sterile syringe and mixed with from 4 to 6 parts of sterile physiologic salt solution; from 0.5 to 1 cc. is placed in sterile ampules, which are closed completely and heated in a water bath for two hours at 60 C. and again for one hour the following day. The fresh as well as the heated material must be sterile in aerobic and anaerobic cultures. The sterile antigen should give a positive reaction in persons with inguinal lymphogranulomatosis and negative reactions in normal persons and in patients with soft chancre. The test is made by injecting 0.1 cc. of the turbid fluid intracutaneously. Two control persons are given injections at the same time. The result is read after forty-eight hours. If the result is positive, there will be an inflammatory infiltration at least 0.5 cm. in diameter, frequently larger, and commonly surrounded by a red area. Sometimes a small area of necrosis develops in the center. The infiltration can be felt by palpation. In the control persons there must be no trace of infiltration. The positive result signifies that the patient in question suffers from or has suffered from inguinal lymphogranulomatosis. The reaction turns out positively in a large majority of infected or previously infected persons.

DEMONSTRATION OF ELEMENTS IN TISSUES: II. GOLD AND SILVER. WALTHER and WERNER GERLACH, Virchows Arch. f. path. Anat. **282**:209, 1931.

A spectroscopic method of analysis is presented which reveals minute traces of these elements. In a case of pulmonary tuberculosis treated with sodium gold thiosulphate, the Christeller zinc chloride method of demonstrating gold in tissues was positive in only a few organs. By the spectroscopic method almost every tissue in the body was found to contain the metal. Silver, too, can be demonstrated in the same way. The method is rapid and certain, and can be applied for other elements also.

PERRY J. MELNICK.

PYRIDINE-SODA METHOD FOR IMPREGNATION OF MESOGLIA AND RETICULO-ENDOTHELIAL CELLS. W. K. BELEZKY, Virchows Arch. f. path. Anat. **282**:214, 1931.

An accurate silver impregnation method is given for demonstrating mesoglia and also reticulo-endothelial cells, suitable for gelatin and celloidin sections. The procedure is varied slightly to adapt it to normal brains, brains of embryos and brains of experimental animals. An attempt is made to eliminate the empiricism of similar methods, by adjusting the p_H of the solutions to produce the proper physicochemical reactions.

PERRY J. MELNICK.

DOUBLE-BLADED SAW FOR BONE. A. FELLER and F. WINDHOLZ, Virchows Arch. f. path. Anat. **284**:640, 1932.

The authors describe an ingenious double-bladed saw for the preparation of thin slabs of bone. They have found it of great value in roentgenographic study of bone.

O. T. SCHULTZ.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

Regular Monthly Meeting, Dec. 12, 1932

PAUL R. CANNON, *President, in the Chair*

STAINING OF OLIGODENDROGLIA AND MICROGLIA IN PYROXILIN (CELLOIDIN) SECTIONS. ARTHUR WEIL and H. A. DAVENPORT.

The methods of staining oligodendroglia and microglia in pyroxilin (celloidin) sections which we shall describe are advantageous for routine work because of their simplicity. The methods are modifications of that devised by J. B. Stern (*Ztschr. f. d. ges. Neurol. u. Psychiat.* **138**:769, 1932), which allow a more exact titration of the ammoniacal silver than in the original method.

In both modifications, oligodendroglia as well as microglia are stained, the one more prominently than the other. For routine work we prefer the method for microglia.

Staining of Microglia.—1. Wash pyroxilin sections 15 microns thick in distilled water.

2. Stain the sections for from fifteen to twenty seconds in the following solution: To 2 cc. of concentrated ammonia (28 per cent) add 10 per cent silver nitrate drop by drop from a buret (approximately 18 cc.), shaking the solution in order to prevent the formation of a precipitate. The end-point of the titration is a slight opalescence of the solution. If through an excess of silver nitrate a precipitate has been formed, or if in the formaldehyde solution a heavy precipitate forms on the section, 1 drop of concentrated ammonia is added, and again the solution is titrated with 10 per cent silver nitrate to the correct point, i. e., the first appearance of a slight opalescence.

3. Transfer a section to 15 per cent formaldehyde, moving it rapidly until a coffee-brown color has been produced. The formaldehyde should be renewed for each section.

4. Transfer the section through three changes of distilled water.

5. Dehydrate in alcohol, clear in xylene and mount in balsam. The sections should be mounted immediately, because keeping them in xylene results in fading.

Staining of Oligodendroglia.—1. Wash the sections in distilled water and transfer to water containing 1 drop of concentrated ammonia per ten cubic centimeters.

2. Stain the sections for from fifteen to twenty seconds in silver solution which has been prepared as described, except that 15 per cent silver nitrate has been used. Approximately 12 cc. of it is added to 2 cc. of concentrated ammonia, until the end-point of the titration has been reached.

3. Transfer a section to 10 per cent formaldehyde and allow it to drop to the bottom of the dish without moving it. After the pyroxylin is darkly stained and the tissue begins to take a brown color, move it with a glass rod until it is stained coffee-brown. Use fresh formaldehyde for each section.

4 and 5. The final steps are as described for the staining of microglia.

DISCUSSION

P. C. BUCY: Some of my results with this stain have been satisfactory, and others not.

A. WEIL: The unsatisfactory results seem to come with an excess of silver nitrate in the reagent. To such a reagent add an excess of ammonia and retitrate with silver nitrate solution.

CHORION EPITHELIOMA OF THE TESTICLE WITH METASTASES, GYNECOMASTIA AND PREGNANCY REACTION (FRIEDMAN). G. W. SCHELM, J. G. DICKSON and G. E. STAHR.

An enlisted sailor, aged 33, entered the Naval Hospital, Great Lakes, Ill., on Oct. 6, 1932, because of severe lumbar backache, weakness, loss of weight and swelling of the right testis to the size of a lemon. An enlarged left supraclavicular lymph node was excised and found to contain tumor tissues, which were diagnosed as metastatic chorion epithelioma. The mammary glands were enlarged and had the appearance of those of a girl at puberty. The Friedman modification of the Ascheim-Zondek test was positive. Death occurred recently, but the results of the postmortem examination are not available at this time.

GUMMA OF THE HYPOPHYSIS AND HYPOTHALAMUS. E. B. FINK.

The article will be published in full in the ARCHIVES.

LEIOMYOSARCOMA OF THE JEJUNUM. J. RUSSELL BRINK and GRANT H. LAING.

About 250 sarcomas of the small bowel have been recorded, and of these approximately from 6 to 8 per cent are myogenous. Stammler's compilation (in v. Bruns, P.: Neue deutsche Chirurgie, Stuttgart, F. Enke, 1924, chap. 5) cited 14 leiomyosarcomas of the small bowel, and since then 5 more have been described. As with other tumors, the etiology is obscure. The growths have occurred in adults, aged from 39 to 73 years. Usually the upper portion of the small intestine (duodenum and jejunum) is the site of growth. The tumor extends out from the wall of the bowel as a large subserous mass without stenosis of the lumen, or into the lumen causing partial occlusion. Of the growths on which the data are given, 13 were subserous and pedunculated, and 5 extended in a polypoid manner into the lumen of the bowel. They do not encircle the bowel and cause a saccular dilatation of the wall as do the lymphosarcomas. Such differences of growth suggested to Demel (*Virchows Arch. f. path. Anat.* **269**:160, 1928) that the tumors growing into the lumen arise from the muscularis mucosa, and that those extending beneath a serous membrane arise from the muscularis propria. Other opinions propose that the tumors arise from an indifferent matrix with myoblastic potentials.

The myogenous character of the spindle-shaped cells in the tumor tissues is demonstrated by suitable stains. Many reports of spindle cell sarcoma of the small bowel are without such studies. Structurally, these growths are interwoven bundles of slender spindle cells with elongated, sinuous nuclei, mingled with a moderately abundant fibrous stroma. The growth expands slowly, and metastases develop late. Because of its insidious and symptomless progress, metastases usually are present when the primary growth is discovered and excised. Demel reported the surgical excision of a metastasis to the liver and the postmortem disclosure two years later of a primary leiomyosarcoma in the jejunum. When the growth extends in a polypoid manner into the lumen of the bowel, there are delayed emptying of the stomach, regurgitation of bile, belching and colicky pains, tarry stools and even acute obstruction. These symptoms appear early, and for this reason such a tumor can be entirely excised. The symptoms when the growth is external are: (1) obscure weakness and cachexia; (2) sudden syncope with tarry stools, and (3) a palpable abdominal mass. Roentgen examination may not demonstrate the growth, and a bleeding gastric or duodenal ulcer may be diagnosed. Absence of occult blood in the stools between attacks of tarry bowel movements does not rule out leiomyosarcoma. Torsion obstruction of the bowel and necrosis of the tumor tissues are complications.

W. D., a white man, aged 41, entered St. Luke's Hospital on Feb. 24, 1932, with a history of an urgent, soft, tarry bowel movement the previous night and two similar bowel movements a few hours before admission. Following these he was weak, but had no acute pain. During July, 1930, he had had a similar attack and was treated in another hospital for four weeks. This controlled the slight

substernal discomfort that he had. When admitted, he had a slight anemia, but was not acutely ill. In the right lower quadrant of the abdomen was a firm, nodular, movable tumor about 12 cm. in diameter. This, as shown by roentgenograms, compressed the transverse and ascending colon, but did not occlude the lumen of the small or large bowel. There were 3,700,000 red cells and 7,750 white cells per cubic millimeter of blood. The urine was unchanged. On Feb. 29, 1932, Dr. H. E. Jones removed a large tumor of the jejunum, which compressed the transverse colon, and also 6 inches (15 cm.) of the transverse colon and 2 inches (5 cm.) of jejunum. Four days after operation the patient's temperature rose to 103 F., the abdomen became distended, and on March 9, 1932, he died of acute peritonitis.

The ovoid tumor, to which 4.5 cm. of small bowel and 11.5 cm. of transverse colon were attached, was 15.5 cm. wide, 10 cm. long and 5 cm. thick. It was flattened dorsoventrally, and the surfaces were elevated by nodules as large as 3.7 cm. These nodules were covered by thin, gray fibrous tissue. A segment of jejunum on the left anterior surface was 4.5 cm. long and 5.5 cm. in circumference, and in a region 2 cm. in diameter was directly continuous with the tumor. About the margin, the plicae circulares were prominent, and the mucosa was gray-yellow and granular, but in the center there was a firm, gray-yellow, slightly elevated tissue. The wall of the intestine not invaded by tumor was 0.4 cm. thick; that invaded was 2 cm. thick. On surfaces made by cutting, two thin membranes continuous with the outer bowel surfaces extended into the tumor tissue 2 cm., and ended abruptly, leaving a region 1.6 cm. wide in which the tumor tissue was continuous. Attached to the inferior border of the tumor by thin fibrous and fat tissues of the transverse mesocolon was 12 cm. of transverse colon, 5.5 cm. in diameter. The mucosa was gray-brown and had no ulcers or hemorrhages. The wall was 0.4 cm. thick. A portion of the lesser omentum, 22 cm. long and 14 cm. wide, and moderately laden with fat, was firmly adherent to the anterior surface of the colon and tumor by fibrous adhesions. This was easily stripped off. The broad surfaces made by cutting the tumor had regions of gray-tan fibrillar tissue and liquefied regions filled with blood-stained necrotic material.

The tumor consisted of bundles of spindle cells with oval nuclei having evenly scattered chromatin and rounded ends. Mallory's stain demonstrated the noncollagenous character of the tumor tissues and staining properties similar to those of smooth muscle. The van Gieson, Weigert and phosphotungstic acid-hematoxylin stains demonstrated further the noncollagenous character of the tumor cells. Fibrous tissue septums divided the tumor into irregular regions.

The chest and abdomen were examined post mortem by Edwin F. Hirsch. The essentials of the anatomic diagnosis were: acute generalized serofibrinous peritonitis; metastatic leiomyosarcoma of the liver, mesenteric lymph nodes and peritoneum; recent surgically incised wound of the abdomen; recent lateral anastomosis of the transverse and ascending colon and of two loops of jejunum; necrosis and pericolonic abscess of the colonic anastomosis.

Leiomyosarcoma of the small bowel is rarely diagnosed clinically, mainly because of its insidious course. Cattell stated that patients of a cancer age who have duodenal stasis, as proved by roentgen examination, and occult blood in the stools should have an abdominal exploration in the absence of anything to account for this condition. In cases of unexplained melena, a sarcoma should be considered.

DISCUSSION

EMIL RIES: Four years ago a woman came to me because of severe hemorrhages from the bowel. Roentgen examination of the gastro-intestinal tract failed to disclose an abnormality. During an exploratory laparotomy, a tumor, 3 by 1 by 1 inches (7.5 by 2.5 by 2.5 cm.), resembling the one described, was found in the wall of the jejunum just below the ligament of Trietz and was removed. The patient is living and well. The tumor growth was antimesenteric, and the lining of the bowel was smooth except for a small depression.

Book Reviews

Individuality of the Blood in Biology and in Clinical and Forensic Medicine. By Prof. Leone Lattes, Director of the Institute of Forensic Medicine in the University of Modena. Translated by L. W. Howard Bertie, M.A., B.M., B.Ch. (Oxon.), from the French Edition of 1929, Thoroughly Revised and Brought up to Date by the Author. Price, $7.50. Pp. 413. New York: Oxford University Press, 1932.

This book, which was first published in Italian in 1923, was translated into German and enlarged by Schiff in 1926 (reviewed in ARCH. PATH. **1**:165 [Jan.] 1926) and was revised and translated into French in 1929; it has been brought up to date by the author and is now published for the first time in the English language. As the author states in his preface, the original work is no longer recognizable because of the extraordinary advances that have been made in this subject in recent years.

The first chapter deals with the nature of the iso-agglutination reaction and various phenomena that have been confused with it. It is pointed out that the blood group factors are not restricted solely to the red blood cells, but are also present in almost every tissue of the body, and also in tumors. Furthermore, the blood group is constant throughout life, once it has been established, and is not affected by disease. The subdivision of human beings has been increased by the discovery by Landsteiner and Levine of the agglutinogens M, N and P. The existence of subgroups of group A (first described by von Dungern and Hirschfeld in 1910) is attributed by the author, like Thomsen, to two quantitatively different A receptors, in contradistinction to Landsteiner's theory that the difference is a qualitative one. The existence of individual blood differences in lower animals is also briefly dealt with.

The author has collected data concerning the heredity of the Landsteiner blood groups, totaling 8,717 families with 19,089 children. Tables summarizing the results of each author since the original observations by von Dungern and Hirschfeld are given. Lattes (like all other authorities at present) agrees that the data satisfy the Bernstein theory of heredity, so that the original theory of von Dungern and Hirschfeld and the alternative theory of linkage proposed by Bauer and others must be given up. The percentage of exceptions to the theory has markedly fallen in recent years because of improvement of technic. A certain minimum must be expected, however, because of illegitimacy. Data on the heredity of the agglutinogens M and N of Landsteiner and Levine, involving 375 families with 1,234 children, has also been accumulated. The results show complete agreement with the theory of heredity proposed by Landsteiner and Levine.

Tables showing the variation in distribution of the blood groups in various populations are given in the fourth chapter. The agglutinogens M and N also vary in their distribution in different populations, studies having been made thus far on Americans, American Indians, Germans, Japanese, Belgians, French, Danes, Poles and Italians. The results of these studies have given rise to various theories of the origin of the races, and blood group determination must take its place now along with other racial differences in anthropologic studies.

In the fifth chapter a brief history of transfusion is given, and the causes and nature of transfusion reactions are discussed. It is pointed out that when donors are selected by the serologic method, according to the Landsteiner four blood group scheme, fatal reactions are practically completely eliminated, provided the technic of performing the transfusion is satisfactory. Besides routine typing, routine cross-matching is advised as a check on the typing and also because of the possible existence of anomalous powerful agglutinins, particularly as a result of repeated transfusions with the same donor. Keeping lists of donors of each

group is advised, and the use of the "universal donor" is advised only in cases of emergency when a donor of the same group as the patient is not available, provided the donor has no powerful agglutinins (is not a "dangerous universal donor"). The agglutinogens M, N and P and the subgroups of A seem to play no part in transfusion reactions, so that for practical purposes Landsteiner's original scheme of four blood groups may be adhered to. Thus far, the application of blood grouping to skin grafting has yielded only indifferent success, possibly because of the defective technic of grafting.

The blood groups have two distinct applications in forensic medicine: (1) in affiliation cases and (2) for the individual diagnosis of blood stains. It has received its greatest application for the purpose of proving nonpaternity in Germany (where Schiff estimated their number at 5,000 in 1929) and in Austria (the number of cases was estimated at 1,500 by Werkgartner). It is also being used to some extent in Russia, Denmark, Norway, Sweden, the Netherlands, Japan, Brazil, Great Britain, the United States and Italy. The time seems ripe also for the introduction of the agglutinogens M and N for the purpose of proving nonpaternity; a combination of the two methods will make it possible to prove nonpaternity in one of three cases, when the putative father is not the true father. The technic of making blood group diagnoses on blood stains, first suggested by Landsteiner and Richter, and fully developed by Lattes, is described, and the possible forensic applications are discussed. It is now possible also to make blood group diagnoses on corpses, seminal stains, small amounts of blood (such as that from a squashed flea) and small amounts of saliva (such as that on the gummed edge of an envelop).

The final chapter deals with the technic of blood grouping, and is followed by a complete bibliography of almost 100 pages (2,375 titles). The book should have the effect of acquainting both the medical and the legal profession with the many possible applications of this work in their respective fields.

A Handbook of Experimental Pathology. By George Wagoner, M.D., Associate in Pathology, and R. Philip Custer, M.D., Associate in Research Pathology, School of Medicine, University of Pennsylvania. Price, $4, postpaid. Pp. 141, with 22 illustrations. Springfield, Ill.: Charles C. Thomas, 1932.

A foreword by E. B. Krumbhaar explains the usefulness of such a book as this written in English and emphasizes the meticulous care of animals, asepsis and general anesthesia essential to those who conduct experimental work. The first section of the book, comprising thirty-eight pages, gives an outline of surgical, physiologic and other experimental methods, anesthesia and general care of animals, with charted complete data of the normal blood findings in all varieties of animals likely to be used. The description of experiments in general pathology comprises forty-five pages, devoted to circulation, retrogressive changes, inflammation, regeneration and repair, infection and immunity and a brief discussion of the possibilities of experiments concerning tumors.

The experiments in special pathology, described in forty-four pages, cover cardiovascular, hemolytopoietic, reticulo-endothelial, respiratory, gastro-intestinal, genito-urinary, skeletal and endocrine systems, followed by sections of nine pages on vitamins and hypersensitiveness. There are an index of experiments arranged in accordance with the plan of the book and a general index. The illustrations are in the form of line and wash drawings.

The series of experiments is adequate for a course far beyond the limits possible in most schools. Nevertheless, it is an excellent list, from which can be selected those experiments made possible by time and equipment in any given instance. The technical procedures are described in detail sufficient for the direction of any one even slightly experienced in this type of study. They are clear and concise and are supplemented by excellent illustrations. At the end of most of the experiments is a series of questions such as are found in the usual student laboratory guides. These are well chosen, but the hazards of success in single experiments

are such that a description of results would be more in keeping with the conception of a handbook. Especially valuable to any experimental worker are the charts, which give in great detail the cytology, chemistry and physical chemistry of the blood in animals.

In only a few instances may any exception be taken to the experiments recommended. The physiologic experiments on aortic valve lesions are so arranged as to be recovery experiments. Certainly one of the most valuable features of the production of aortic stenosis is the demonstration to the students of the murmur, which is possible only with a wide open thorax and is not practicable as a recovery experiment. Interrupted artificial respiration is not described, and is useful in open thorax work because of the respiratory wave in the blood pressure tracings. For acute experiments on aortic insufficiency the Wiggers cannula gives better results than valve hooks. The study of air embolism is enhanced by record of the blood pressure and respiration. The experiments on infection are excellent, but those on immunity can be found in more elaborate form in other books. Experimental anaphylaxis in birds would be a valuable addition to the other experiments on this subject.

The proof reading was excellent, but an occasional error is noted, such as "metaphysical osteoid tissue" and "Fahreaux" for "Fahraeus."

The study of pathology has been and still is based on abnormal morphology. This, however, of itself can give no comprehensive and rational understanding of disease. The best program is that which builds on the foundation of morbid form a conception of how the changes have developed and how they are associated with abnormal function. This can best be achieved by the experimental studies so well displayed in this book. This was well understood by Virchow, reached a high degree of development in the hands of Cohnheim and has been wisely extended and soundly exploited in this country by W. G. MacCallum, R. M. Pearce, Jr., and others. The reviewer is convinced that this book is a noteworthy addition to the literature of pathology and will be of great aid to teachers of pathology and clinical medicine who wish to give their students an understanding of the processes of disease and to inspire in them a fruitful curiosity as to its cause, development and manifestations.

The Heart Rate. By Ernst P. Boas, M.D., Associate Physician, Mount Sinai Hospital, New York, and Ernst F. Goldschmidt, Ph.D. Cloth. Price, $3.50. Pp. 166, with 68 illustrations. Springfield, Ill.: Charles C. Thomas, 1932.

This monograph is based on a study of the heart rate in three hundred and fifty-six persons by means of the Boas cardiotachometer, which counts the heart beats continuously for long periods. There are two sections: One is devoted to the heart rate of normal persons, and the other to the heart rate of sick persons. A brief but interesting history of pulse mensuration is found in the first chapter. The cardiotachometer is described in detail. The authors present studies on a small group of normal persons included in a rather narrow age range and distributed among few occupations. These studies give a much more satisfactory standard for the heart rate of normal persons at work, rest and sleeping than have hitherto been available. The consideration of the heart rate during sleep is probably the most important contribution in the book. The clinical section is devoted to the heart rate during surgical operations and in a variety of disorders of the circulation. The use of the cardiotachometer in all the studies so far made has provided sound confirmation of the already current views of physiologist and clinician—opinions or traditions often based on none too satisfactory data. There is a bibliography of one hundred and eighty references.

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